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Part A: Workshop on Winter Flounder Biology, 1986
Part B: Workshop on Winter Flounder Biology, 1989
Part C: Winter Flounder Biology Workshop, 1991
Part D: Flatfish Biology Workshop, 1994
Part E: Flatfish Biology Workshop, 1996
Part F: Flatfish Biology Conference, 1998
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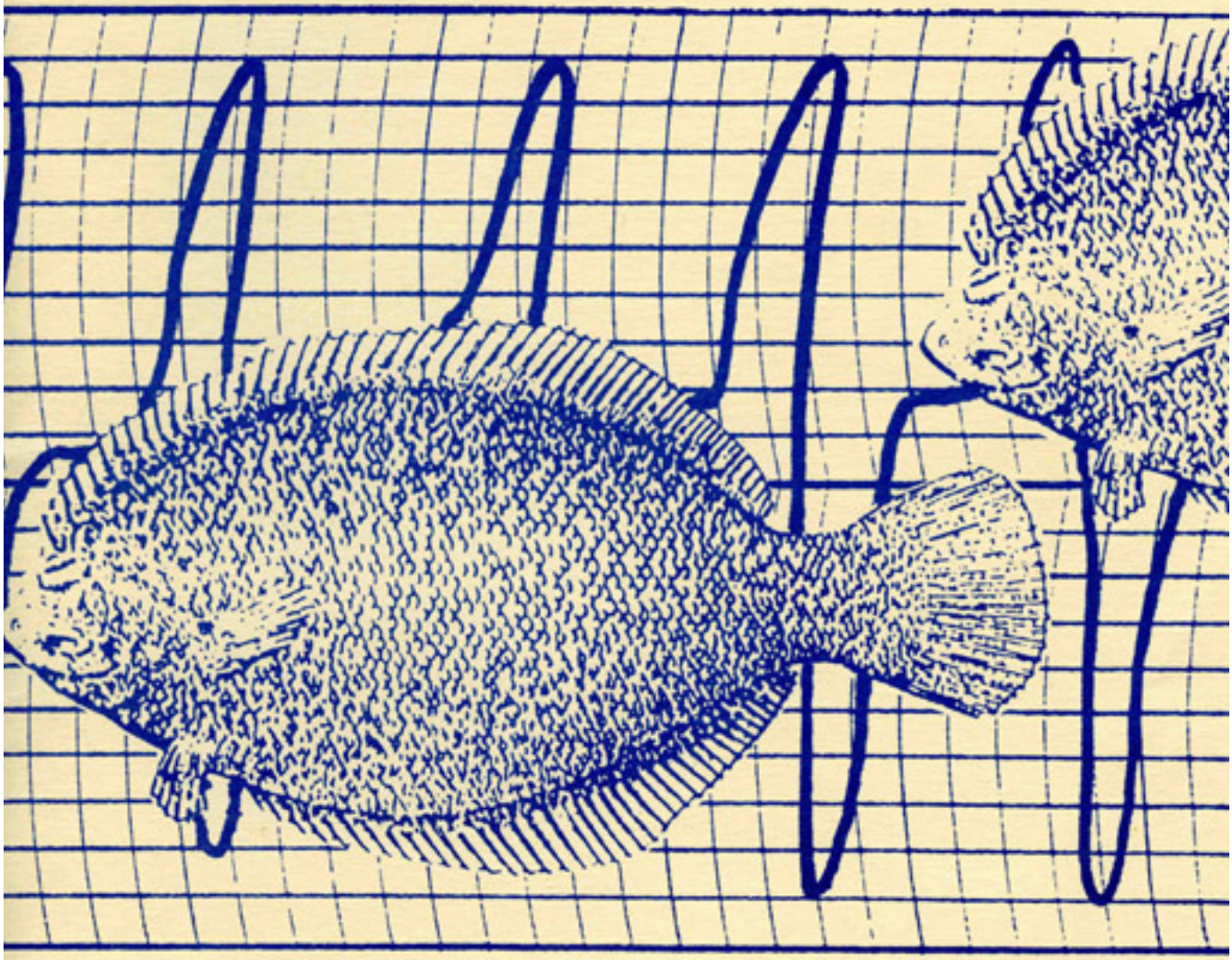
Renee Mercaldo-Allen and Anthony Calabrese, Editors

February 2008

Workshop On

WINTER FLOUNDER BIOLOGY



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Northeast Fisheries Science Center Reference Document 08-05a

Workshop on Winter Flounder Biology
December 2-3, 1986, Mystic, Connecticut

by Conference Steering Committee: Anthony Calabrese (Chair)[1], Allan Beck[2], Steven Clark[3], Arnold Howe[4], Ambrose Jearld[3], Chris Powell[5], Eric Smith[6], and Anne Studholme[7]

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First in a series of Flatfish Biology Conferences

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National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts

February 2008

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Milford CT

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Woods Hole, MA

Workshop on Winter Flounder Biology

December 2-3, 1986, Ramada Inn, Mystic, Connecticut

Oral Presentations

Tuesday, December 2

8:30 a.m. **Registration**

10:00 a.m. Welcome and Introduction
Alan Peterson, Jr., Director
Northeast Fisheries Center
Woods Hole, MA

Anthony Calabrese, Conference Chair
Northeast Fisheries Center
Milford, CT

10:20 a.m. Individual Summary Statements
Winter Flounder Biology Research in the Northeast

Eric Smith (Chair)
Connecticut Department Environmental Protection
Waterford, CT

1:00 p.m. **Lunch (no-host)**

Session I: Life History Studies

Steven Clark, Chair
Northeast Fisheries Center
Woods Hole, MA

2:00 p.m. Abundance, Growth and Mortality of Juvenile Winter Flounder in the Lower Niantic River, CT, from 1983 Through 1986.
D. J. Danila
Northeast Utilities Environmental Laboratory, Waterford, CT

2:30 p.m. Growth of Winter Flounder in Three Areas of Long Island Sound
P. Howell
Connecticut Department of Environmental Protection, Waterford, CT

3:00 p.m. Winter Flounder Occurrence in Lower New York Harbor
A. L. Pacheco
Northeast Fisheries Center, Sandy Hook, NJ

3:30 p.m. **Coffee and Poster Set-up**

Session I (Continued)
Arnold Howe, Chair
Massachusetts Division of Marine Fisheries
Sandwich, MA

4:00 p.m. Structuring of an Estuarine Fish Community by Climatically-induced Population Changes in the Winter Flounder, *Pseudopleuronectes americanus*
P. Jeffries
University of Rhode Island, Kingston, RI

4:30 p.m. Tidal and Diel Behavior of Larval Winter Flounder in the Niantic River Estuary, CT
J. D. Miller
Northeast Utilities Environmental Laboratory, Waterford, CT

5:00 p.m. **Poster Set-Up**

5:30 p.m. **Hosted Mixer and Poster Session**

Christopher Powell, Chair
Rhode Island Division Fish and Wildlife
Kingston, RI

Wednesday, December 3

8:00 a.m. **Registration**

Session II: Larval Studies

Ambrose Jearld, Chair
Northeast Fisheries Center
Woods Hole, MA

8:30 a.m. Development of the Early Otolith Record in Winter Flounder
M. F. Davis[1, 2], A. Jearld[2], and S. Sass[3]
[1]Fort Valley State College, Fort Valley, GA, [2]Northeast Fisheries Center, Woods Hole, MA, and [3]Massachusetts Division of Marine Fisheries, Sandwich, MA

9:00 a.m. Phenotypic Variation in Size and Age at Metamorphosis in Winter Flounder, *Pseudopleuronectes americanus*
R. C. Chambers and W.C. Leggett
McGill University, Montreal, Quebec

9:30 a.m. Reproductive Success of the Winter Flounder in Long Island Sound
J. B. Hughes, D. A. Nelson, D. M. Perry, J. E. Miller, G. R. Sennefelder, and J. J. Pereira
Northeast Fisheries Center, Milford, CT

10:00 a.m. **Coffee**

Session III: Metabolic Studies

Anne Studholme, Chair
Northeast Fisheries Center
Sandy Hook, NJ

10:30 a.m. Sulfate Excretion in Winter Flounder *(Pseudopleuronectes americanus):* Control by Glucocorticoids
J. L. Renfro and L.E. Barber
University of Connecticut, Storrs, CT

11:00 a.m. Detection of Biochemical Effects in Winter Flounder from Coastal Massachusetts
J. J. Stegeman, F. Y. Teng, and E. A. Snowberger
Woods Hole Oceanographic Institute, Woods Hole, MA

11:30 a.m. Impact of an Ocean Sewage Outfall on Winter Flounder: Biochemical and Histopathological Studies
R. E. Hillman, R. S. Carr, and J. M. Neff
Battelle New England Research Laboratory, Duxbury, MA

12:00 p.m. Essential Amino Acid Absorption by Flounder: The Effects of Mercury Compounds
A. Farmanfarmaian, K. Pugliese, V. Iannaccone, and V. Klimek
Rutgers University, Piscataway, NJ

12:30 p.m. **Hosted Buffet Lunch**

Session IV: Pathology and Distribution

Alan Beck, Chair
U. S. Environmental Protection Agency
Narragansett, RI

1:30 p.m. Pollution-associated Biological Effects in Boston Harbor Winter Flounder
R. Murchelano
Northeast Fisheries Center, Woods Hole, MA

2:00 p.m. Development of Vacuolated Cells in Diseased Liver of Winter Flounder from Boston Harbor
J. E. Bodammer[1] and R. A. Murchelano[2]
[1]Northeast Fisheries Center, Oxford, MD, and [2]Northeast Fisheries Center, Woods Hole, MA

2:30 p.m. Epitheliocystis Lesion in Gills of the Winter Flounder
(*Pseudopleuronectes americanus)*
E. J. Lewis[1], J. J. Ziskowski[2], and T. K. Sawyer[3]
[1]Northeast Fisheries Center, Oxford, MD, [2]Northeast Fisheries Center, Highlands, NJ, and [3]RESCON Associates, Royal Oak, MD

3:00 p.m. Thames River (CT) Winter Flounder Migration
D. Tolerlund
U.S. Coast Guard Academy, New London, CT

3:30 p.m. **Adjourn**

Poster Session

Christopher Powell, Chair
Rhode Island Division Fish and Wildlife
Kingston, RI

Tuesday, December 2, 5:30 p.m.

Biomonitoring Methods Using the Winter Flounder
D. Black
U.S. Environmental Protection Agency, Narragansett, RI

Growth and Maturation of Winter Flounder from the Mid-Atlantic, Southern New England, Gulf of Maine and Georges Bank Regions
J. Burnett
Northeast Fisheries Center, Woods Hole, MA

The Life Cycle and Pathology of *Glugea stephani* in Winter Flounder
A. Cali and P. M. Takvorian
Rutgers University, Newark, NJ

Fluorescent Monitoring of Bacterial Ingestion by Phagocytic Leucocytes in Winter Flounder, *Pseudopleuronectes americanus*
T. G. Daniels
University of Rhode Island, Kingston, RI and Science Applications International Inc., Narragansett, RI

Adult Winter Flounder Population Abundance Surveys in the Niantic River, Connecticut
D. J. Danila
Northeast Utilities Environmental Laboratory, Waterford CT

Histopathologic Lesions of Winter Flounder from Northeast Estuaries: Results from the First Two Years of the National Status and Trends Program
S. Y. Everline, J. J. Evans, and M. W. Newman
Northeast Fisheries Center, Oxford, MD

Tumor Development in Winter Flounder Exposed to Contaminated Marine Sediment under Laboratory and Field Conditions
G. R. Gardner[1], P. P. Yevich[1], A. R. Malcolm[1], P. F. Rogerson[1], L. J. Mills[2], A. G. Senecal[3], T. C. Lee[3], J. C. Harshbarger[4], and T. P. Cameron[5]
[1]U.S. Environmental Protection Agency, Narragansett, RI, [2]Science Applications International Corporation, Narragansett, RI, [3]University of Rhode Island, Kingston, RI, [4]Smithsonian Institution, Washington, D.C. and [5]National Cancer Institute, Bethesda, MD

Egg and Larval Studies on Winter Flounder from a Pollution Gradient in Long Island Sound
A. T. Hebert, J. E. Miller, and D. M. Perry
Northeast Fisheries Center, Milford, CT

The Immune Response of a Marine Teleost, *Pseudopleuronectes americanus* (Winter Flounder), to the Protozoan Parasite *Glugea stephani*

R. Laudan[1], J. S. Stolen[2], and A. Cali[1]
[1]Rutgers University, Newark, NJ, and [2]Northeast Fisheries Center, Highlands, NJ

Physiological Monitoring of Winter Flounder from Polluted and from Relatively Non-Polluted Sites in Long Island Sound

J. Pereira (Field-study Coordinator)
Northeast Fisheries Center, Milford, CT

Hepatic Tumors and Other Liver Pathology in Massachusetts Flatfish

S. L. Sass[1], R. A. Murchelano[2] and T. Currier[1]
[1]Massachusetts Division Marine Fisheries, Sandwich, MA, and [2]Northeast Fisheries Center, Woods Hole, MA

Regulation of Xenobiotic and Steroid Metabolism by Estradiol in Winter Flounder

E. A. Snowberger and J. J. Stegeman
Woods Hole Oceanographic Institution, Woods Hole, MA

Glugea stephani Disease in American Winter Flounder (*Pseudopleuronectes americanus*) Populations

P. Takvorian and A. Cali
Rutgers University, Newark, NJ

Gross Observations of Winter Flounder Lesions from Long Island Sound and Other Northwest Atlantic Sites

J. Ziskowski[1], M. Newman[2], and R. Murchelano[3]
[1]Northeast Fisheries Center, Sandy Hook, NJ, [2]Northeast Fisheries Center, Oxford, MD, and [3]Northeast Fisheries Center, Woods Hole, MA

Abstracts
Oral Presentations

Abundance, Growth, and Mortality of Juvenile Winter Flounder in the Lower Niantic River, CT, from 1983 Through 1986

D. J. Danila

Northeast Utilities Service Company
Northeast Utilities Environmental Laboratory
PO Box 128, Waterford, CT 06385

With the exception of Pearcy's work in the Mystic River, CT during 1958-60, little has been published concerning the abundance, growth, and mortality of post-larval young-of-the-year winter flounder using field data. Northeast Utilities Environmental Laboratory (NUEL) has collected data to estimate these parameters at several stations in the lower Niantic River from 1983 through 1986. The data have provided additional information for the assessment of the impact of Millstone Nuclear Power Station on this local stock of winter flounder. The study fills the gap between the end of larval development, when mortality rates are high and growth is rapid, and the juvenile period at age 1 and older, when growth is slower and mortality is low. These abundance data also may be used as an index of year-class strength.

A 1-m beam trawl designed by NUEL was used with four interchangeable nets (0.8- to 6.4-mm mesh) to collect juveniles just after metamorphosis from late May through September. Two stations were sampled, one near the river mouth (LR) and one about 900 m upriver (WA). Both stations were in shallow (1-1.5 m) water near the shoreline and each was sampled once a week during daylight within about 2 h before to 1 h after high tide. The beam trawl was hauled for 50 to 100 m, depending upon the abundance of juveniles and three (1983) or four (1984-86) replicate tows were taken at each station.

Growth of young varied between the two stations and among years. Growth was significantly greater at station LR than at WA during all years and flounder averaged about 20 mm larger there by the end of September. At LR, growth was greatest in 1983 (to about 70 mm), decreased to similar levels in 1984, and 1985 (65 mm) and was significantly less in 1986 (50 mm). Annual growth patterns at WA were less variable among years and means were similar (ca. 45 mm) in September. Growth was inversely related to abundance at LR, with lowest densities found in 1983, similar numbers in 1984 and 1985, and the highest densities in 1986. Densities at WA were more variable, but were also greatest in 1986. Apparent monthly mortality estimates (actual mortality plus emigration off station) were determined from annual catch curves and were not greatly different from year to year (43-44%) at LR. Mortality was apparently not related to either density or growth rate. Mortality estimates using this method were not reliable at WA because of the highly variable weekly density estimates. Presently, the relationship between density and growth is being examined, as well as different mortality estimation procedures. The effects of temperature on the length of the larval season, time to metamorphosis, and on the growth rate of juvenile flounder are also under study.

Growth of Winter Flounder in Three Areas of Long Island Sound

P. Howell

Connecticut Department of Environmental Protection
Marine Fisheries Division
Waterford, CT 06385

Investigations of Long Island Sound winter flounder have historically found differences in growth patterns seen in the western Sound versus the eastern Sound. Catches taken from a trawl survey of Long Island Sound during 1984-86 also showed that the length frequency of winter flounder taken from the western sound was significantly smaller than lengths of eastern samples. Standardized catches taken during the first two weeks of June from eastern (east of the Connecticut River), western (west of Stratford Shoals), and central Long Island Sound were compared. Length frequencies (200-299 mm total length) were balanced so that the three samples were identical. Increase in weight with length was determined by log-log regression and the slopes compared by one-way ANOVA. The resulting length-weight relationships indicated that the central and western fish had a greater rate of increase in weight with length (slope), and a lower Y-intercept. A similar comparison of August catches resulted in no difference in the length-weight relationship among the three areas. These data therefore give no evidence that winter flounder from the western Sound are less robust than those taken from the eastern Sound. Since the shorter length frequency of western samples reflects a shorter length-at-age, possible explanations for the slowed growth might be less than optimal temperature regimes in the central and western Sound, and/or adverse conditions in the nursery grounds early in the life history of the western populations.

Winter Flounder Occurrence in Lower New York Harbor

A. L. Pacheco

National Marine Fisheries Service
Northeast Fisheries Center
Highlands, NJ 07732

A fishery habitat utilization study was conducted for the Corps of Engineers in 1981-1983. Winter flounder were the most ubiquitous fish species. Size and seasonal occurrence at the various stations are summarized and related to fishery concerns in coping with contaminated dredge spoil management in the metropolitan area.

Structuring of an Estuarine Fish Community by Climatically-induced Population Changes in the Winter Flounder, *Pseudopleuronectes americanus*

P. Jeffries

University of Rhode Island
Graduate School of Oceanography
Kingston, RI 02281

Weekly trawl samples taken over the last 21 years with standardized precision in Narragansett Bay and Rhode Island Sound show major changes in population size of the most numerous species. Fifty-fold increases in annual catch of a migratory species component appear to be inversely related to an 83% reduction in Narragansett Bay's year-round resident winter flounder population, which is statistically, if not as yet functionally, correlated with winter-spring climatic warming. Since the resident winter flounder and migratory populations share the same benthic-epibenthic food sources, nutritional structuring of the entire complex is suggested. However, other populations of lesser numerical importance have also experienced significant but unrelated change in size and scope of their occurrence in the Narragansett Bay area. Nutritional factors, climate, disease, and predation apparently are involved in these cycles of abundance. The cyclic nature of each undoubtedly produces within the system resonant disturbance over the long term that could be confused with short-term, pollutant-induced mortality.

Tidal and Diel Behavior of Larval Winter Flounder in the Niantic River Estuary, CT

J. D. Miller

Northeast Utilities Service Company
Northeast Utilities Environmental Laboratory
PO Box 128, Waterford, CT 06385

Larval winter flounder have been sampled during their seasonal occurrence in the Niantic River since 1983. Samples were taken twice weekly at three stations in the Niantic River and one station in Niantic Bay to monitor changes in temporal and spatial abundance. Collections were made with a 60-cm bongo sampler towed in a stepwise oblique pattern. Net mesh size was 202 µm during and 333 µm after the occurrence of yolk-sac larvae. Four 24-h studies were conducted at one station in the river to examine tidal and diel behavior. Stationary collections were made at the mouth of the river during eight tidal cycles to measure tidal transport.

Early developmental stages (ca. 45 mm and smaller), prior to fin ray development, behaved as passive particles with no diel difference in sampling density and a net export from Niantic River to Niantic Bay. These young larvae appeared in the bay approximately one month after becoming abundant in the river, which agreed with hydrographic calculations of average particle retention time in the Niantic River. Later developmental stages (ca. 4.5 mm and larger) showed both a tidal and diel behavioral response. In areas of weak tidal currents, sample densities were highest in night collections. When strong tidal currents were present, larvae were more prevalent in flood tidal stage collections. Also, there was a net import of these larger larvae from the bay to the river. Apparently, later developmental stages migrate vertically in conjunction with tidal currents to reenter and remain in the river.

Development of the Early Otolith Record in Winter Flounder

M. F. Davis[1, 2], A. Jearld, Jr.[2], and S. Sass[3]

[1]*Fort Valley State College, Biology Department*
Fort Valley, GA 31030

[2]*National Marine Fisheries Service*
Northeast Fisheries Center, Woods Hole, MA 02543

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18 Route 6A, Sandwich, MA 02563

A continuing sequence of research studies on the effects of environment and physiology on the formation of subannual increments in winter flounder otoliths was begun. Correlation between behavioral, anatomical, and physiological changes was attempted, as well as the gathering of preliminary data on daily growth increments.

Otoliths were removed from embryonic through year-old laboratory raised and young-of-the-year wild caught winter flounder. Daily growth increments were counted from photographs taken on both light and scanning electron microscopes (SEM). Behavioral observations were made from hatching through metamorphosis.

Growth equations for groups of laboratory-raised larvae were found to be linear with no inflection point at 20-30 days. Ring counts from otoliths prior to 28 days post-hatch did not fit a linear pattern. These increments were qualitatively different from later increments. Increment counts for ages from 28-55 days post-hatch fit a linear regression line with approximately one increment being laid down per day. Sagitta size and fish length relationships for two groups were exponential for premetamorphic larvae but linear by the end of the first year. Pearson correlation coefficients were calculated for two other groups of larvae. The correlation between fish length and otolith dimension for these groups was very weak for the younger larvae, but became much stronger for the older larvae. The correlation was strongest between otolith length and preserved standard length (0.88) and even stronger when younger and older larvae were combined (0.98).

Linear regression lines and R^2 values were calculated comparing age and otolith dimensions for two groups of larvae. Otolith dimensions were highly variable and age alone did not account for most of this variability in younger larvae. In older larvae, age accounted for a greater amount of variability, at least for sagitta length (R^2=0.705). However, when both larval groups were pooled, the R^2 values for all otolith dimensions at age were very high with R^2 for otolith length being highest (0.97).

The major behaviors associated with winter flounder metamorphosis involved the transfer from pelagic to benthic habits and anatomical transformation to asymmetrical form. These included change in swimming method from side-to-side tail-whip in pre-metamorphic larvae to undulating fins in juveniles, and change in feeding from more active and more frequent lunging to less active and less frequent gulping. Amount of time spent resting on the bottom increased as amount of time swimming decreased. Overall post-metamorphic juveniles maintained lower activity levels than pre-metamorphic larvae.

Phenotypic Variation in Size and Age at Metamorphosis in Winter Flounder, *Pseudopleuronectes americanus*

R. C. Chambers and W. C. Leggett

McGill University, Department of Biology
1205 Ave. Dr. Penfield, Montreal, Quebec H3A 1B1

Winter flounder, like all flatfish, undergoes a dramatic morphological, behavioral, and ecological metamorphosis between its larval and juvenile stages. The timing of and size at transition between the pelagic larval and demersal benthic stages are variable within populations. Prerequisites for inferences about the relevance of this variation include determining its amount, pattern, and sources. Our results on these prerequisites are the focus of this paper.

During June of 1986, adult flounder from a population in Conception Bay, Newfoundland, were collected and their gametes stripped. A paternal half-sib mating design was employed so that the observed variation in age and size at metamorphosis of the progeny could be partitioned into maternal and paternal sources. Full-sib groups were reared in triplicate laboratory populations. A total of 22 laboratory populations were followed through the entire larval period, generating a total of 674 observations.

Length of metamorphosis was less variable than age at metamorphosis (coefficient of variation= 6.2 and 13.3, respectively), even though temperatures were held constant (8ºC) for the entire period. Length and age at metamorphosis were weakly, though significantly, correlated ($r= 0.42$, $P <0.0001$). This means that individuals that metamorphosed later were relatively large at metamorphosis. This relationship held both within populations and across population (family) averages. Growth rate (average daily increment in length) and developmental rate (l/(age at metamorphosis)) were strongly correlated ($r= 0.78$, $P< 0.0001$). Although individuals that metamorphose relatively late are larger than those that metamorphose early, their growth rates are lower (i.e., they are smaller at age). This result also has a within and between population component.

We conclude that developmental rates, which determine when a larva exits the pelagic habitat, are highly variable and are under parental influence. Variation in these rates may be maintained by fluctuating selection pressures due to variable larval habitats.

Reproductive Success of the Winter Flounder (*Pseudopleuronectes americanus*) in Long Island Sound

J. B. Hughes, D. A. Nelson, D. M. Perry, J. E. Miller, G. R. Sennefelder, and J. J. Pereira

National Marine Fisheries Service
Northeast Fisheries Center
Milford, CT 06460

A study was initiated to measure the reproductive success of the winter flounder (*Pseudopleuronectes americanus*) collected from six sites in Long Island Sound along a (reported) pollution gradient. This effort focused on larval hatchability and development, cytogenetic analysis of embryos, and levels of pollutants in pre- and post-fertilized fish eggs. Bottom water from the sites was used in all standard crosses. Standard crosses of one female x pooled sperm of three males were made with fish collected at four sites. At the remaining two sites a modified dominant lethal gene test was conducted (each cross consisting of one female x one male) and fertilizations were made of fish from a presumed non-impacted site fertilized in bottom water from an impacted site and vice versa. At spawning, samples were taken of pre-and post-fertilized eggs for PCB and heavy metal analyses. Samples for cytogenic study were taken at the 16-cell, blastula, tail-bud, and post-hatch stages. Hatchability estimates were based on samples taken 1-2 days post-hatch. Results of the hatchability assay indicate no statistically significant differences in egg samples from the six stations. Cytogenic analysis of blastula-stage embryos for mitotic abnormalities indicated a significant difference ($P<0.01$) between crosses made with Hempstead fish and crosses of Shoreham fish, with Hempstead flounder embryos having a higher rate of mutation. Preliminary cytogenetic data indicate a moderate impact at the other four stations. Dominant lethal crosses analyzed thus far indicate no significant differences (one-way ANOVA and Scheffe tests) between males. Analysis of mitotic rate of tail-bud embryos from Hempstead, Shoreham, and Madison fish indicates Madison embryos nearly 50% more active than those from Hempstead and 20% more active than those from Shoreham.

Sulfate Excretion in Winter Flounder (*Pseudopleuronectes americanus*): Control by Glucocorticoids*

J. L. Renfro and L. E. Barber

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Department of Physiology and Neurobiology
Storrs, CT 06268

Winter flounder regularly ingest seawater, which contains a high concentration (25mM) of sulfate. The sulfate absorbed by the intestine is excreted almost exclusively by the kidneys, via the process of renal secretion. We have studied this secretory process at three levels of organization-intact kidney, isolated renal tubule epithelium, and isolated tubule cell brush border and basolateral membrane vesicles. Regulation of secretion was examined by determining the relationship of sulfate at the above three levels in flounder acclimated for at least three weeks to 100% SW and SO_4-free 10% SW.

A sulfate:anion exchanger is present in renal tubule brush border membrane vesicles (BBMV) prepared by a calcium precipitation method from flounder acclimated to 100% SW. A 21-mm HCO_3 gradient, in>out, produced concentrative $^{35}SO_4$ uptake (overshoot) at 15s, which was 3- to 4-fold higher than equilibrium (60 min). A 100-mM NaSCN gradient, out>in, produced a ^{3}H-glucose over-shoot at 1 min about 2.5-fold equilibrium. BBMV isolated from SO_4-free 10% SW showed NA-dependent glucose overshoot not significantly different from BBMV isolated from 100% SW fish. However, a 21-mM HCO_3 gradient, in>out failed to produce concentrative SO_4 uptake. A 100-mM NaCL gradient , out>in, compared to 100-mM NaCL, out=in, had no significant effect on SO_4 uptake by BBMV from either 100% or 10% SW animals. Renal sulfate clearance ratios were determined in 10% SW fish before and after intravenous infusion of sodium sulfate sufficient to increase plasma sulfate concentration 3- to 4-fold. ^{3}H-polyethylene glycol was used as glomerular filtration marker. The sulfate clearance ratio averaged 0.71 ± 0.16 (SE) during infusion of isosmotic mannitol, indicating net sulfate reabsorption. However, following 2 h of fusion of isosmotic sodium sulfate plus mannitol the clearance ratio was 1.81 ± 0.16, indicating net secretion. Thus, the presence of the renal brush border anion exchange membrane transporter was associated with the ability of the kidney to perform net sulfate secretion.

To evaluate possible regulatory factors, we have examined the effects of glucocorticoids (dexmethansone or hydrocortisone phosphate) on sulfate transport. Daily administration of 30-µg/100 g bd. wt. for five days produced a highly significant 2.5-fold increase in the sulfate transport rate by BBMV from flounder acclimated to SO_4-free 10% SW. In primary monolayer cultures of flounder renal tubule epithelium, hydrocortisone phosphate produced a 3-fold increase in net sulfate secretion compared to monolayers cultured in the absence of this hormone.

*Supported by the National Science Foundation

Detection of Biochemical Effects in Winter Flounder from Coastal Massachusetts

J. J. Stegeman, F. Y. Teng, and E. A. Snowberger

Woods Hole Oceanographic Institution
Biology Department
Woods Hole, MA 02543

Levels of hepatic microsomal monooxygenase activity were consistent with induction of cytochrome P-450 by environmental chemicals in winter flounder (*Pseudopleuronectes americanus*) from several sites along coastal Massachusetts. Levels of activity were higher in fish from Boston Harbor and Plymouth Bay than in fish from Nantucket Shoals and Buzzards Bay. There was a close correlation between levels of EROD and AHH activities in fish from the latter site, with some individuals there showing little evidence of induction. Immunoblot analysis of flounder liver microsomes with a monoclonal antibody (1-12-3) against the B-naphthoflavone (BNF)-inducible cytochrome P-450 isozyme (P-450E) from the marine fish scup, revealed a single cross-reacting protein in untreated fish from all four field sites. A similar protein was induced in BNF-treated winter flounder. We conclude that this protein is the flounder counterpart of cytochrome P-450E, and the data indicate environmental induction of this flounder isozyme. Levels of monooxygenase activity in Buzzards Bay fish correlated positively with the amount of flounder cytochrome P-450E counterpart, supporting the conclusion that there was a wide range in the extent of induction in animals in Buzzards Bay. Induced cytochrome P-450 could function in activating environmental carcinogens, a probable factor in the tumorigenesis in winter flounder in Boston Harbor. Monoclonal antibodies as employed here should be useful in further analysis of such induced cytochrome P-450 in these and other fish.

Impact of an Ocean Sewage Outfall on Winter Flounder: Biochemical and Histopathological Studies

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Winter flounder, *Pseuopleuronectes americanus*, from Boston Harbor near the Deer Island sewage outfall and from a nearby reference population near Plymouth Beach, Massachusetts, were collected on several occasions and the livers analyzed for a variety of biochemical parameters and histopathological conditions. Flounder livers contained a variety of lesions, including necrotic foci, aggregations of macrophage cells, hepatocyte vacuolation, hyperplastic and neoplastic growths. Lesions in the livers were more prevalent in the Boston Harbor population than in the reference population.

A number of biochemical parameters in winter flounder tissues, including hepatic ascorbic acid, glycogen, and lipid concentrations, and the concentration ratios of free amino acids in somatic tissue were significantly different between the two populations. Highly significant statistical associations were observed between the presence of presumptive preneoplastic cells in livers of winter flounder from Boston Harbor and low hepatic ascorbic and glycogen concentrations. The biochemical differences between the Boston Harbor and the reference populations were pronounced in individuals in which histopathological lesions were prevalent.

Essential Amino Acid Absorption by Flounder: The Effects of Mercury Compounds*

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Estuaries of the Mid-Atlantic region of the United States are the site of spawning and early development for the commercially and recreationally important summer flounder, *Paralichthys dentatus*, and winter flounder, *Pseudopleuronectes americanus*. Many of these estuaries receive effluents from industrial, commercial and domestic sources, resulting in their pollution by classes of contaminants that include heavy metals. Mercury compounds are among the most potentially hazardous of heavy metals. They accumulate through the food chain and appear in the gastrointestinal tract of larger fish in high concentrations. The interaction of mercury with the brush border membrane of the intestinal epithelium interferes with the normal digestive and absorptive functions of the intestine. In the present study the effects of $HgCl_2$ and CH_3HgCl on the intestinal uptake of the essential amino acid L-leucine were examined in the summer and winter flounder. Fish from relatively pristine and polluted estuaries of the Mid-Atlantic region of the U. S. were compared in this respect.

The results indicate that species differences in uptake rates for L-leucine exist; these species also show differential sensitivity to $HgCl_2$ and CH_3HgCl when challenged. $HgCl_2$ is the more potent inhibitor of leucine uptake. There also appears to exist a differential in the unchallenged uptake rates and the percent inhibition resulting from challenge when summer flounder from pristine and polluted areas are compared. Chronic exposure in polluted embayments appears to reduce the uptake of essential amino acids.

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Pollution-associated Biological Effects in Boston Harbor Winter Flounder

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Anthropogenically-derived inorganic and organic chemical contaminants can be found in estuaries and in coastal waters adjacent to large population centers throughout the U. S. Although it frequently is alleged that some contaminants affect the well-being of marine organisms, including commercially important resource species, substantiation of specific biological effects as caused by specific contaminants remains a difficult task. Morphological abnormalities in tissue architecture- histologic lesions-have been widely used to evaluate the health of marine fishes. Unfortunately, however, most histological lesions are not adequately specific to implicate particular contaminants as inducers. Integumental ulcers (fin rot) and hepatocarcinoma (liver cancer) both are promising lesions for evaluating the effects of environmental contaminants on fish health. Hepatocarcinoma, in several flatfish species particularly, is a consequential disease which appears related to the presence of organic contaminants in sediments and trophically important biota. Recent studies have disclosed high levels of polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and other organic contaminants in sediments of Boston Harbor. Winter flounder are abundant in the harbor and are commercially and recreationally important. Biochemical studies of inducible enzyme systems responsible for the metabolism of organic molecules have revealed that winter flounder have high levels of mixed function oxydases (MFO). Histopathologic studies of 325 Boston Harbor winter flounder have disclosed that approximately 25% have hepatocarcinoma. The lesions observed resemble those experimentally induced in rodents and may be anthropogenically-derived organic chemical contaminants.

Development of Vacuolated Cells in Diseased Liver of Winter Flounder From Boston Harbor

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Light and electron microscopic studies were conducted on liver lesions of winter flounder (*Pseudopleuronectes americanus*) that demonstrated grossly visible tumors, as previously described by Murchelano and Wolke (1985). Areas (lesions) containing large numbers of vacuolated cells were studied and, based on their affinities with surrounding parenchymal cells, are believed to be of hepatocyte origin. Unlike normal appearing liver cells, the vacuolated ones were arranged in ductular or acinar configurations, their nuclei were apically located, and their cytoplasm contained variably-sized inclusion bodies, which were also present in their vacuoles. In all cases where vacuolated cells were prominent, the typical distribution of the sinusoids was absent, suggesting that these tissues may have been hypoxic.

Ultrastructural observations indicated that the large vacuoles of these tissues resulted from dilated endoplasmic reticulum (ER) cisternae, and their prominent inclusion bodies contained mainly profiles of degenerate mirochondria and other cytoplasmic components. The groups of vacuolated cells were surrounded by connective tissue and numerous fibroblasts. Parenchymal cells adjacent to the vacuolated cell foci appeared shrunken or slightly necrotic, and those with condensed cytoplasm were undergoing changes in which portions of their mitochondria and ground substances appeared to pinch off from the main body of the cell.

These observations, particularly the dramatic distended ER cisternae, suggest the effects of hepatotoxin(s), and the extrusive loss of cytoplasm and organelles from cells via inclusion bodies is a principal feature of apoptotic cell death.

Epitheliocystis Lesions in Gills of the Winter Flounder (*Pseudopleuronectes americanus*)

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Winter flounder, *Pseudopleuronectes americanus*, were collected from nearshore waters along the northeast coast of the United States from Maine south to New Jersey, and offshore in the vicinity of Georges Bank during the years 1982-1985. Epitheliocystis-like lesions occurred in 290/950, or 31% of the fish examined. The number of lesions per 6- µm section ranged from 1 to 155, with rare occurrences of more than 20 lesions per section.

Lesions in representative gill tissue were examined with transmission electron microscopy and specifically identified as epitheliocystis disease by the presence of Chlamydia-like bodies.

Seasonal data showed that prevalence of infection was higher in the winter, with a level of 49% in the January-March quarter, than in the summer, with a low level of 11% in the July-September quarter.

Length data showed that the prevalence was twice as high in fish larger than 20 cm (36%) than in fish less than 20 cm (17%).

Average percentages of the infection in fishes sampled over the 3-year period were found to vary from 22% in the New York Bight area compared to 44% in the vicinity of Cape Cod, Massachusetts, and in Long Island Sound.

These data when published will be of value to other researchers conducting comparative studies documenting diseases, host ranges, host specificity and distribution, and mechanisms of disease transmission in marine fishes. Furthermore, the data provided, when integrated with additional life history, cytological, and biological information, should prove useful in resolving the systematics of this perplexing group of organisms. The data also suggest there is some potential for these organisms to be used as biological tags in population dynamics studies.

Thames River (CT) Winter Flounder Migration

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A small-scale (about 2,000) fish, long-term (1975-84) study of movements of the winter flounder (*Pseudopleuronectes americanus*) within and from the Thames River estuary was conducted using spaghetti tags. Based upon a 150-tag return (7.6% recovery), it was observed that this species is very provincial or territorial. Of the 23 returns from beyond one mile of the mouth of the river, only 4 traveled west, not getting beyond the Niantic River. The other 19 flounder were found as far east as Narragansett Bay (RI). This strongly suggests that the flounder spawned in the Thames River support the commercial fisheries of Fishers Island and Block Island Sound.

Poster Abstracts

Biomonitoring Methods Using the Winter Flounder

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Several *in situ* biomonitoring methods are being developed using the winter flounder, *Pseudopleuronectes americanus.* Contaminated study areas include Gaspee Point in upper Narragansett Bay, RI, New Bedford Harbor in Buzzards Bay, MA, noted for its PCB contamination, and Apponagansett Bay, MA, a less contaminated site near New Bedford. Fox Island, a relatively clean area in lower Narragansett Bay, serves as a reference area. Although adult flounder disperse offshore during the summer, a tag and recapture study verified their annual residence and exposure to contaminants at Gaspee Point during the spawning season: a similar migratory pattern was assumed for Buzzards Bay fish. Biomonitoring methods being evaluated include adult morphometry, tissue residues, and embryo/larval growth and survival. Results indicate that adult female flounder from Gaspee Point have significantly higher hepatosomatic indices, but significantly lower gonadosomatic indices than Fox Island fish. Morphometry was not examined in Buzzards Bay fish. Growth, survival and contaminant residues were measured in the progeny of fish collected from each study area. Eggs from New Bedford Harbor flounder contained significantly higher concentrations of PCB (39.6 µg/g dry weight), and larvae that hatched from these eggs under clean laboratory conditions were significantly smaller in length (2.96 mm) and weight (22µg). Although contaminated, Apponaganett Bay and Gaspee Point larvae were not significantly different in size from Fox Island larvae.

Future work will examine morphometry of Buzzards Bay winter flounder, cytochrome P-450 induction, and investigate further larval growth, including measurement of RNA/DNA ratios.

Growth and Maturation of Winter Flounder from Mid-Atlantic Southern New England, Gulf of Maine, and Georges Bank Regions

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Preliminary growth and maturity analyses were conducted for 14,030 winter flounder collected from the mid-Atlantic, southern New England, Gulf of Maine, and Georges Bank regions during Northeast Fisheries Center bottom trawl cruises from 1975-1984. The von Bertalanffy growth model was fitted to length at age data using nonlinear techniques; maturity at length and age was evaluated by probit analysis.

In all regions, males grew faster than females but attained a smaller maximum size. Results for the mid-Atlantic, southern New England, and Gulf of Maine regions were difficult to interpret due to offshore mixing of winter flounder with various estuarine-specific growth patterns. Generally, asymptotic lengths (L inf) increased from south to north but the reverse was true for Brody growth coefficients (K). L inf and K values for Georges Bank winter flounder were 53.3 cm total length and 60.4 cm and 0.32 and 0.23 for males and females, respectively.

Length at 50% maturity (L_{50}) and age at 50% maturity (A_{50}) for the mid-Atlantic, southern New England, and Gulf of Maine regions also increased from south to north (21.8 cm and 1.7 years to 25.0 cm and 2.5 years for males, and 23.4 cm and 1.9 years to 25.9 cm and 2.2 years for females). Corresponding values of L_{50} and A_{50} for Georges Bank winter flounder were 22.8 cm and 1.6 years and 28.4 cm and 2.4 years for males and females, respectively.

The Life Cycle of *Glugea stephani* in Winter Flounder

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The microsporidian *Glugea stephani* is a spore-forming intracellular obligate protozoan parasite that infects the intestinal connective tissue of winter flounder. The spore, which is ingested, injects its sporoplasm into a host cell by eversion of a tubular polar filament apparatus. Once inside the host cell cytoplasm, the infective sporoplasm undergoes a series of proliferate cell divisions followed by sporogony. During the parasites' proliferative cycle, the host cell undergoes massive hypertrophy to produce a single-cell hypertrophy tumor, which often exceeds 5 mm in diameter. The proliferative stages of the parasite undergo multiple karyokinetic divisions, during which the nuclear membranes remain intact. Cytokinetic division is delayed and, consequently, multinucleate plasmodia are formed. Eventually, these plasmodia undergo cytokinesis and form numerous uninucleate cells. The uninucleate cells then undergo a series of sporogenic karyo-and cytokinetic divisions, accompanied by alteration of their plasma membrane. These uninucleate cells undergo a metamorphosis to become spores. They are in sporogeny vacuoles, which are devoid of host cytoplasm. The eventual consequence of the infection is production of mature spores inside greatly enlarged single-celled hypertrophy tumors (xenomas).

Experimental infections resulted in mortality due to one of three types of pathological condition: a low-grade infection with xenomas located in the muscosa, resulting in rupture of the epithelial lining; total disruption of the integrity or occlusion of the intestinal lumen; massive infection of the serosa, resulting in starvation and emaciation.

Fluorescent Monitoring of Bacterial Ingestion by Phagocytic Leucocytes in Winter Flounder, *Pseudopleuronectes americanus**

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A fluorescent staining technique was developed as part of a monitoring procedure designed for potential use to assess the impact of chemically contaminated sediments on the cellular immune system of winter flounder. This *in vitro* technique is used to simultaneously monitor the phagocyctic activity and the killing ability of leucocytes in winter flounder. Head kidney excised from adult flounder (16 and 29 cm) served as a source of leucocytes. Kidney cells were separated using continuous Percoll density gradient centrifugation. Monolayers of adherent granulocytic and non-granulocytic cells were allowed to form on glass slides. Sixty-five percent of the adherent cells demonstrated macrophage-like morphology using light microscopy. Adherent cells were incubated for 2 hours with the bacterium *Vibrio anguillarum* in solutions of acridine orange (AO) and crystal violet (CV). Viable bacteria fluoresce green and nonviable bacteria fluoresce red after phagocytosis. Fluorescent staining is used routinely in mammalian systems to monitor bacterial phagocytosis. The methodology developed here represents the first application to fish immunological studies for use with environmental pollution assessments. In addition, the intracytolasmic granuoles of granulocytes were also made distinguishable with AO as bright red fluorescing bodies and greatly enhanced differentiation of these cells from glass-adherent phagocytes.

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Adult Winter Flounder Population Abundance Surveys in the Niantic River, Connecticut

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As part of the assessment of impact of the Millstone Nuclear Power Station, Northeast Utilities Environmental Laboratory has studied various aspects of the life history and population dynamics of the local Niantic River winter flounder stock. Each year since 1976 we have estimated the abundance of adults spawning in the river from late February through early April. These estimates can be used for the determination of a stock-recruitment relationship and in our stochastic population dynamics model for impact assessment. During the spawning season, adults were captured in the estuary using a 9.1-m otter trawl. All specimens larger than 15 cm (1976-82) or 20 cm (1983-86) were measured, categorized by sex and reproductive condition, and marked with a letter or number made by a liquid nitrogen-chilled brass brand. The brand was changed weekly and all fish marked previously and recaptured were remarked with the current brand. The mark and recapture data were used with the Jolly model for open populations to obtain weekly population abundance estimates. An annual index of abundance was computed by averaging the weekly estimates, after excluding the less reliable first and last values. The catch-per-unit-effort (CPUE) for a standardized trawl tow for flounder larger than 15 cm was also calculated each year over a 4-week period from mid-March thru early April. Since the CPUE data were not normally distributed and were positively skewed, the annual median CPUE and 95% CI was used as a second index of population abundance.

Over the 11-year period, the number of adults marked ranged from 6,820 in 1981 to 2,790 in 1986; recaptures varied from 469 in 1981 to 170 in 1985. The Jolly abundance index showed that the population of Niantic River winter flounder has fluctuated within about a fivefold range (ca. 9,700 in 1986 to 49,500 in 1982). Changes in population were not random but occurred in a cyclic pattern with a decrease seen from 1976 through 1978, an increase to a maximum in the early 1980's, and a decline to the present low level of abundance.

Similarly, CPUE varied from 12.0 in 1986 to 43.4 in 1981 and 42.6 in 1982. This measure of abundance generally followed the Jolly index until 1982, but recently less correspondence was seen between the two. Standardization of trawl tow length in 1983 lessened the variability of CPUE, which may be seen in the smaller confidence interval about each median in recent years. The Jolly abundance indices were less precise and had relatively large CI. Our sampling intensities were sufficiently low (3 to 5.4%) that errors in estimating abundance using the Jolly model most likely range from 25 to 50%. We are currently investigating other factors that may have contributed to the disparity between the two abundance indices. We also have recently attained newer computer programs specifically designed to estimate abundance and to test assumptions and fits of data to the Jolly model.

Histopathologic Lesions of Winter Flounder from Northeast Estuaries: Results from the First Two Years of the National Status and Trends Program

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The Benthic Surveillance Project of NOAA's National Status and Trends Program seeks to determine the level of organic and inorganic contaminants in sediments and fish tissues and their biological effects on target species. More than 50 locations in the nation's estuaries and other nearshore waters are being sampled to explore possible correlations between contamination and biological effects.

Findings from the first year of sampling in the northeast region show that sediments from 4 to 12 sites (Raritan Bay, Western Long Island Sound, Boston Harbor, and Salem Harbor) contained substantially elevated concentrations of organic contaminants. Distributions of four lesions- giant cells in the tubular epithelium of the kidney, necrotic granulomatous lesions in the kidney, proliferation of macrophage centers in the kidney, and biliary hyperplasia in the liver-paralleled the distributions of elevated sediment contaminant levels. A definite diagnosis of neoplasia was made only on livers of Boston Harbor flounder although lesions suspected as being pre-neoplastic were observed at other locations.

Tumor Development in Winter Flounder Exposed to a Contaminated Marine Sediment under Laboratory and Field Conditions*

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A collaborative EPA/NCI project is in progress to: (1) evaluate the carcinogenic potency of contaminated marine sediment in laboratory and field-exposed flounder, and (2) identify and assess potentially causative agents through a combination of chemical analysis and short-term, biological testing of sediment extracts. Chemical characterization of the test sediment indicates contamination by polychlorinated biphenyls, aliphatic hydrocarbons and cycloalkanes, dichlorodiphenyldichlorethylene (DDE), some two-ring aromatic hydrocarbons (acenaphthene, acenaphthylene, biphenyl, and naphthene), many polycyclic aromatic hydrocarbons (fluorene, phenanthrene, dibenzothiophrene, fluoranthene, pyrene, chrysene, benzo(a)pyrene, benzo(e)pyrene, perylene, and various alkylated derivatives of these compounds). Other identified compounds include dichlorodiphenyltrichloroethane (DDT), Chlordane, Ethylan, ketones, quinones, carbazoles, and pthalate esters. Identified inorganic elements include arsenic, cadmium, chromium, copper, lead, manganese, and nickel. Some of these substances are known to be genotoxic, carcinogenic, co-carcinogenic, and tumor promoting. Results to date are preliminary. In laboratory-exposed fish, (4 months to 0-1 year class and 3 months for 1 year class) neoplastic disease was observed in kidney, pancreatic islets, oral and esophageal epithelia and odontogenic tissue. Although histopathological evaluation of fish collected from the Black Rock Harbor study site is incomplete, a hepatic neoplasm and cyctic lesions of the spleen have been identified in field-collected winter flounder. At a second contaminated site (New Bedford Harbor, Massachusetts), a high prevalence of hepatomas and a renal neoplasm have been identified in field-collected animals. Pancreatic adenomas have been identified in fish collected from New Bedford Harbor and in fish exposed in the laboratory to Black Rock Harbor sediments. Extracts of sediments from Black Rock Harbor have been evaluated in the Ames test for mutagens and in the Chinese hamster V79 metabolic cooperation assay for tumor promoters. Test results confirm the presence of both mutagens and tumor promoters in the sediment. Relationships between tumor development and the presence of tumor-enhancing agents in test sediments are being explored.

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Eggs and Larval Studies on Winter Flounder from a Pollution Gradient in Long Island Sound

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Illustrations are given of the techniques involved in the collection, dissection, and staining of winter flounder (*Pseudopleuronectes americanus*) eggs for cytological and cytogenic analysis. Examples of abnormal development in the embryos, such as cellular de-differentiation and gross morphological anomalies, are shown. Methods of spawning and culturing are also presented.

The Immune Response of a Marine Teleost, *Pseudopleuronectes americanus* (Winter Flounder), to the Protozoan Parasite *Glugea stephani*

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Glugea stephani is an intracellular cyst-forming microsporidian parasite that is found in the intestine of winter flounder (WF), *Pseudopleuronectes americanus*. No detectable humoral response was seen in parasitized fish or in fish injected with either spores or spore homogenate. Quantification of total immunoglobin (Ig) levels showed a decrease in IgM levels, rather than enhancement, 21 days after intramuscular (IM) injections of spores ($3x10^6$/ml). When a second injection of spores was administered on day 21 and tested 3 weeks later, a further decrease in total serum Ig's occurred. A decrease in total IgM levels also occurred in WF that were simultaneously injected with *G. stephani* and the antigens, horse red blood cells (HRBC), or formalin-killed *Klebsiella pneumonia* (KP). The total IgM levels of fish injected with an antigen plus spores were not as low as those injected with the parasite alone. The IgM levels, as well as antibody titers to HRBC and KP, were lower, however, when compared to fish injected only with the HRBC or bacteria. Disrupted spore homogenate, injected into winter flounder, showed a less marked decrease in IgM levels when compared with whole spores. When a single IM injection of spores was given, followed by two weekly injections of indomethacin (a drug that inhibits prostaglandin activity), no decrease in Ig levels occurred and levels were compatible to control (saline-injected) fish.

Physiological Monitoring of Winter Flounder from Polluted and Relatively Non-polluted Sites in Long Island Sound

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An interdisciplinary program, designed to measure the impact of pollutants on the general health and reproductive success of winter flounder, has been undertaken at the NMFS/NOAA Milford Laboratory. Four sites representing differing amounts of pollutant impact have been selected for sampling at monthly intervals. The Shoreham (Long Island) site is an area which was found to have low levels of metals and which has been used as a control site in previous studies. Black Rock Harbor is an impacted area whose sediments have been well characterized as part of a dredging study by the Army Corps of Engineers. It is also the site of a landfill, a sewage treatment plant, as well as some industrial outfalls. Morris Cove (New Haven Harbor) handles heavy commercial traffic associated with the petroleum industry. The Niantic site is an area at which fish have been available year-round in the past, and, therefore, is being used as an area for baseline studies.

At each site, samples are taken by the chemistry, biochemistry and physiology groups for analysis. The chemistry group is responsible for assessing the physical characteristics of the site (salinity and dissolved oxygen) and for determination of PCB and metal concentrations in liver and gonad tissue. Enzyme analysis by the biochemical group allows assessment of the activity in various biochemical pathways. Carbonic anhydrase parallels spermiogenesis, while malic enzyme is essential for fatty acid synthesis in the female gonad. Isocitrate dehydrogenase and pyruvate kinase are both involved in energy production in the glycolytic pathway and can thus be used to monitor its activity. Glucose-6-phosphate dehydrogenase (G6PDH) has been shown to be an indicator of general metabolic stress, while malate dehydrogenase is particularly sensitive to heavy metal-induced stress. In addition, G6PDH provides metabolites necessary for the synthesis of vitellogenin in the liver, from where it is transported through the bloodstream to the developing eggs in the gonad. Vitellogenin has been implicated in the transport of pollutants from the liver to the ovary. The blood is analyzed for vitellogenin by the physiology group.

In addition to vitellogenin, the physiology groups monitors a suite of blood parameters which serve as indicators of stress. These include determinations of hematocrit, hemoglobin, bilirubin and total numbers of red blood cells which may be affected by environmental parameters, such as dissolved oxygen or heavy metals. Sodium, potassium, magnesium, calcium, chloride and total osmolarity are also measured since heavy metals can likewise interfere with osmoregulation. The Physiological Ecology Investigation is also conducting a morphological examination of gills from winter flounder utilizing a scanning electron microscope.

Hepatic Tumors and Other Liver Pathology in Massachusetts Flatfish

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As several recent studies have shown, the prevalence of flatfish liver neoplasms (historically rare in wild fish) is a likely indicator of compromised fish health due to contaminants. Since the liver metabolizes toxins, it is especially susceptible to tissue damage. The recent discovery of a high prevalence of liver neoplasia (8%) in Boston Harbor winter flounder led to a broader investigation. The objective of this study was to determine how pervasive this pathology was in commercially important flounder species within Massachusetts's territorial waters.

During Massachusetts Division of Marine Fisheries May and September bottom trawl surveys in 1985, flounder livers were examined for tissue abnormalities. For this 'coastal' sample, those liver sections that had "gross" (i.e., visible) irregularities were excised for histopathological examination; thus sampling was non-random. Since previous examination of winter flounder (*Pseudopleuronectes americanus*) in the vicinity of New Bedford Harbor (NBH) had revealed some evidence of hepatoxic tissue damage, liver samples collected there were randomly preserved for microscopic examination.

Of all the fish examined in the coastal sample, 5.3% had gross liver lesions of unknown etiology. Subsequent histopathological analysis of these livers revealed several types of liver pathology including inflammatory (focal and multi-focal hepatitis, vasculitis, pericholangitis, macrophage aggregate hyperplasia), proliferative (cholangiofibrosis), and necrotic (focal and multifocal origin) lesions, vacuolar cell lesions (ductal and cells of unknown origin), those presumed pre-neoplastic (foci of cellular alteration –basophilic), and neoplasms. Of 218 yellowtail flounder (*Limanda ferruginea*) livers examined, 20 had gross lesions; microscopically, only four of the 20 were confirmed, and all of these were inflammatory. Gross liver lesions were noted in 35 of 589 winter flounder from the coastal sample, but only 15 had microscopically confirmed lesions. Within these 15 livers, 18 lesions were identified as inflammatory, seven were necrotic, four were vacuolated cell lesions, two were pre-neoplastic, and two were neoplasms. One neoplasm was a hepatocellular adenoma from a fish caught near Plymouth Bay; the other was a cholangiocarcinoma from a fish caught off Boston Harbor.

Of 36 winter flounder from the NBH sample, 19 (53%) had microscopic lesions. Twenty-two inflammatory lesions were noted, along with eleven necrotic, one proliferative, and six vacuolated cell lesions.

Of 191 summer flounder (*Paralichthys dentatus*), 124 windowpane (*Scopthalmus aquosus*), 71 American plaice (*Hippoglossoides platessoides*), and 38 witch flounder (*Glyptocephalus cynoglossus*) examined grossly, only 10 livers had apparent abnormalities and these were microscopically normal.

Conclusions on prevalence are restricted by the non-random nature of the coastal sampling methodology, as well as limitations inherent in the histological techniques utilized in this study. Forty-three percent of the gross lesions observed in coastal winter flounder had microscopically confirmed lesions. Conversely, 29% (7/24) of the NBH livers taken in May which did not have gross lesions did have microscopic ones. Greater accuracy in the estimation of lesion prevalences may be achievable through the exclusive use of random sampling, although it would be more costly to implement. The preliminary results described do suggest, however, that winter flounder inhabiting nearshore areas adjacent to urban harbors in addition to Boston Harbor (e.g., New Bedford Harbor, Plymouth Bay) may have higher prevalences of environmentally-associated hepatic disorders than other populations along the Massachusetts coast. Intensive studies in these areas may be warranted.

Regulation of Xenobiotic and Steroid Metabolism by Estradiol in Winter Flounder

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Hepatic microsomes from winter flounder (*Pseudopleuronectes americanus*) and scup (*Stenotomus chrysops*) metabolized estradiol (E_2) to at least seven products separated by thin layer chromatography. The most prominent product constituted 35% of total metabolites. It co-migrated with authentic 2-OH- E_2 in three different TLC solvent systems and is tentatively identified as 2-OH- E_2. Cytochrome P-450-mediated E_2 2-hydoxylase activity was studied by assessing ^3H release from [2-^3H]- E_2. Normalized to protein, liver weight and body weight, E_2 2-hydoxylase activity was lower in mature female winter flounder than in immature females or mature males. However, E_2 2-hydoxylation normalized to cytochrome P-450 was not sexually differentiated. Microsomal ethoxyresorufin O-deethylase (EROD) activity was lower in female winter flounder than in males, relative to both microsomal protein and cytochrome P-450. Polyclonal antibodies against P-450E, the major aryl hydrocarbon hydroxylase in scup, did not affect E_2 2-hydoxylase activity. In conjunction with other data, these observations suggest that P-450E does not contribute toward microsomal E_2 2-hydoxylation and that this activity is regulated differently from EROD in winter flounder. E_2-treated winter flounder demonstrated lower EROD and E_2 P-450. Reduced EROD activity in treated fish is consistent with reduced EROD activity in mature female flounder, which have elevated serum E_2, and suggests regulation by E_2. However, lowered E_2 2-hydoxylation in E_2-treated flounder is surprising, as activity related to cytochrome P-450 was not sexually differentiated in mature flounder. This may indicate a pathway, unavailable in mature female winter flounder, for E_2 to regulate cytochrome P-450-mediated E_2 2-hydoxylation.

Glugea stephani Disease in American Winter Flounder (*Pseudopleuronectes americanus*) Populations

P. M. Takvorian and A. Cali

Rutgers University
Department of Biological Sciences
Newark, NJ 07102

The American winter flounder presents a major portion of the commercial and sport fishery in the northeastern Atlantic coastal area. Unfortunately, relatively little information is available concerning flatfish diseases and their effects on the fishery. One of the diseases to which flatfishes are susceptible is microsporidosis.

Glugea stephani, a microsporidian (protozoan) parasite of the intestinal tract of the winter flounder, is responsible for substantial losses of winter flounder. At present, little information is available concerning: a) this parasite's biological and financial impact on the flounder fishery, b) geographical distribution of the parasite along the northeast coast, the winter flounder's primary habitat, and c) the environmental factors possibly contributing to geographic sites of high parasite incidence ("hot spots").

Our initial laboratory studies, in which we cycled this organism in prerecruit winter flounder, indicate that the protozoan is responsible for a 60% mortality of infected fish.

Our initial field studies were conducted in the N.Y.-N.J. Lower Bay Complex, where we separated the Bay into two areas that coincided with an east/west partition. A comparison of *Glugea*/site incidence percentages indicates that there is a notable increase in infection in the western part of the Bay. Statistically significant differences between infection in the eastern and western part of the Bay were determined by a G-test of significance ($p=0.02$). These data appear to coincide with the east/west heavy metal deposition in Raritan Bay recorded by Greig and McGrath (1977) during their studies of heavy metals in the sediments of Raritan and N. Y. Lower Bay.

For our present field studies we have established 23 collection locations along the NE coast that we are monitoring for *G. stephani* infection. We plan to compare the infection incidence with environmental factors to identify any variables that may be affecting the disease incidence at these sites.

Gross Observations of Winter Flounder Lesions from Long Island Sound and Other Northwest Atlantic Sites

J. Ziskowski[1], M. Newman[2], and R. Murchelano[3]

[1]*Northeast Fisheries Center, Highlands, NJ 07732*

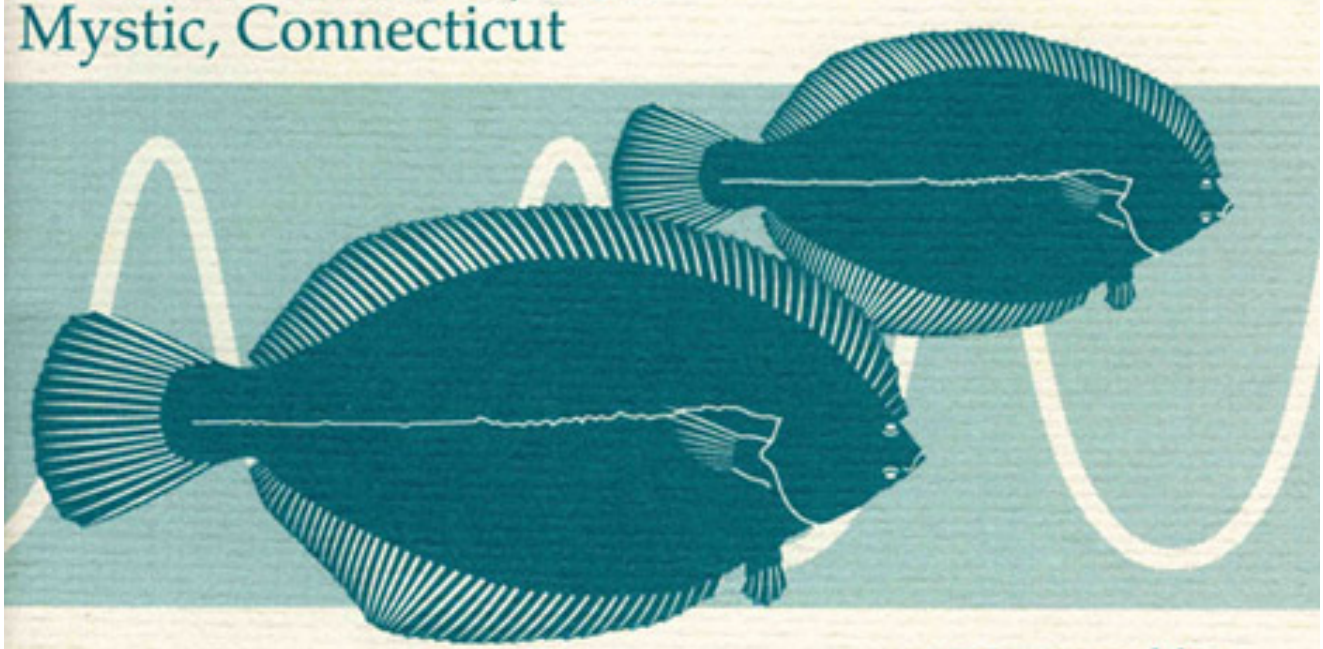
[2]*Northeast Fisheries Center, Oxford, MD 21654*

[3]*Northeast Fisheries Center, Woods Hole, MA 02543*

No Abstract

Workshop on Winter Flounder Biology

December 5 and 6, 1989
Mystic, Connecticut



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Workshop on Winter Flounder Biology
December 5-6, 1989, Mystic, Connecticut

by Conference Steering Committee: Anthony Calabrese (Chair)[1], Allan Beck[2], Steven Clark[3], Donald Danila[4], Arnold Howe[5], Penelope Howell[6], Ambrose Jearld[3], Chris Powell[7], and Anne Studholme[8]

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Second in a series of Flatfish Biology Conferences

U.S. DEPARTMENT OF COMMERCE
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Woods Hole, Massachusetts

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Workshop on Winter Flounder Biology

December 5-6, 1989, Ramada Inn, Mystic, Connecticut

Oral Presentations

Tuesday, December 5

8:00 a.m. **Registration/coffee**

9:00 a.m. Welcome and Introduction
Alan Peterson, Jr., Science and Research Director
Northeast Fisheries Center
Woods Hole, MA

Anthony Calabrese, Conference Chair
Northeast Fisheries Center
Milford, CT

Session I

Allan Beck, Chair
U. S. Environmental Protection Agency, Narragansett, RI
Narragansett Bay Estuarine Research Reserve, Narragansett, RI

9:30 p.m. Invited Summary Statements-Winter Flounder Biology Research in the Northeast

10:30 p.m. Atlantic States Marine Fisheries Commission, Interstate Fisheries Management Program- Update
P. Howell
Connecticut Department of Environmental Protection, Marine Fisheries Division, Waterford, CT

Session II

Penny Howell, Chair
Connecticut Department of Environmental Protection,
Marine Fisheries Division, Waterford, CT

10:45 a.m. The 12-Mile Dumpsite Recovery Study: Preliminary Findings Relative to Winter Flounder
R. Pikanowski, S. Wilk, A. Pacheco, D. McMillan, B. Valdes, J. Rugg, and L. Stehlik
National Marine Fisheries Service, Highlands, NJ

11:45 a.m. A Three-year Assessment of Reproductive Success in Winter Flounder, *Pseudopleuronectes americanus*
D. A. Nelson and J. Hughes
National Marine Fisheries Service, Milford, CT

12:15 p.m. **Lunch (no host)**

Session III

Ambrose Jearld, Chair
National Marine Fisheries Service
Woods Hole, MA

1:30 p.m. Movements and Exploitation of the Niantic River Stock of Winter Flounder
D. J. Danila
Northeast Utilities Environmental Laboratory, Waterford, CT

2:00 p.m. Growth Rates of Juvenile Winter Flounder in Different Estuarine Habitats: a Comparison of Habitat Quality
S. M. Sogard and K. W. Able
Rutgers University Marine Field Station, Tuckerton, NJ

2:30 p.m. Tumorigenesis in Winter Flounder
M. J. Moore, R. M. Smolowitz, and J. J. Stegeman
Woods Hole Oceanographic Institution, Woods Hole, MA

3:00 p.m. **Coffee Break and Poster Set-up**

Session IV

Don Danila, Chair
Northeast Utilities Environmental Laboratory
Waterford, CT

3:30 p.m. Organic Anion Transport by Flounder Renal Proximal Tubule in Culture: Inhibition by 2, 4-D and DDA
M. A. Dawson [1, 2] and J. L. Renfro[2]
[1]National Marine Fisheries Service, Milford, CT and [2]University of Connecticut, Storrs, CT

4:00 p.m. Age Determination of Winter Flounder in Rhode Island Using Sectioned Otoliths
R. E. Haas and C. W. Recksiek
University of Rhode Island, Kingston, RI

4:30 p.m. Phosphate Transport in Winter Flounder Renal Proximal Tubule Primary Cultures
A. Gupta and J. L. Renfro
University of Connecticut, Storrs, CT

5:00 p.m. **Poster Set-up**

5:30 p.m. **Hosted Mixer and Poster Session**
Chris Powell[1] and Don Danila[2], Chairs
[1]Rhode Island Division Fish and Wildlife, West Kingston, RI and [2]Northeast Utilities Environmental Laboratory, Waterford, CT

Wednesday, December 6

Session V

Anne Studholme, Chair
National Marine Fisheries Service
Sandy Hook, NJ

8:00 a.m. **Registration/Coffee**

8:30 a.m. Impacts of Treated Municipal Wastewaters on Development and Growth of Winter Flounder
P. Weis[1], J. S. Weis[2], A. Greenberg[3], and C. M. Chen[3]
[1]UMDNJ-New Jersey Medical School, Newark, NJ, [2]Rutgers University, Newark, NJ, and [3]New Jersey Institute of Technology, Newark, NJ

9:00 a.m. Some Factors Affecting the Abundance and Growth of Larval Winter Flounder in the Vicinity of Niantic, Connecticut
J. D. Miller
Northeast Utilities Environmental Laboratory, Waterford, CT

9:30 a.m. Winter Flounder Young-of-the-Year Growth and Survival in Mesocosm and Field Ecosystems
K. A. Rose[1], L. W. Barnthouse[1], G. Klein-MacPhee[2], B. Sullivan[2], A. Keller[2], D. Danila[3], and J. D. Miller[3]
[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]University of Rhode Island, Narragansett, RI, and [3]Northeast Utilities Environmental Laboratory, Waterford, CT

10:00 a.m. **Coffee Break**

Session VI

Chris Powell, Chair
Rhode Island Division Fish and Wildlife, West Kingston, RI

10:30 a.m. Predicting Temperature-dependent Developmental Rate and Survival in Hatchling Winter Flounder
R. C. Chambers, W. C. Leggett, and G. L. Maillet
McGill University, Montreal, Quebec, Canada

11:00 a.m. Factors Contributing to Variability in Size and Viability of the Eggs and Larvae of Winter Flounder, *Pseudopleuronectes americanus*, Reared in the Laboratory
L. J. Buckley, A. Smigielski, T. Halavik, and G. C. Laurence
National Marine Fisheries Service, Narragansett, RI

11:30 a.m. Condition of Winter Flounder Larvae in Narragansett Bay as Measured by RNA/DNA Ratio
E. Hjörleifsson
University of Rhode Island, Graduate School of Oceanography, Narragansett, RI

12:00 p.m. **Hosted Lunch**

Session VII

Arnold Howe, Chair
Massachusetts Division of Marine Fisheries
Sandwich, MA

1:30 p.m. Trophic Transfer of PAH Metabolites into Winter Flounder
A. E. McElroy[1], J. D. Sisson[1], J. M. Cahill[1], and K. M. Kleinow[2]
[1]University of Massachusetts, Boston, MA and [2]Louisiana State University, Baton Rouge, LA

2:00 p.m. Winter Flounder Movement—A Historical Review and Current Information on Narragansett Bay Winter Flounder Populations
J. C. Powell
Rhode Island Division of Fish and Wildlife, West Kingston, RI

2:30 p.m. The Eye Movement System of the Flatfish: A Model for Studying Adaptation
W. Graf
Rockefeller University, New York, NY

3:00 p.m. Use of Radiopharmaceuticals for Evaluation of Boston Harbor Winter Flounder
P. R. Burns[1, 2], R. Moore[2], R. A. Wilkinson[2], A. J. Fleschman[2], and H. W. Strauss[2]
[1]Suffolk University, Boston, MA, and [2]Massachusetts General Hospital, Boston, MA

3:30 p.m. Habitat Utilization by Winter (*Pseudopleuronectes americanus*) and Smooth (*Liopsetta putnami*) Flounders in Great Bay Estuary, New Hampshire
M. P. Armstrong
University of New Hampshire, Durham, NH

4:00 p.m. **Adjourn**

Poster Session

Tuesday December 5, 5:30 p.m.

Development of Potential Toxicity Indices in Isolated Winter Flounder Hepatocytes
S. M. Baksi[1] and D. H. Campana[2]
[1]U.S. Environmental Protection Agency, Narragansett RI, and [2]Science Applications International Corporation, Narragansett, RI

The Effect of Hypoxia on the Growth of Young-of-the-Year Winter Flounder
A. J. Bejda, B. Valdes, and A. L. Studholme
National Marine Fisheries Service, Sandy Hook, NJ

A Gross and Histological Atlas of Winter Flounder Larvae—Progress to Date
J. E. Bodammer[1] and G. Klein-MacPhee[2]
[1]National Marine Fisheries Service, Oxford, MD, and [2]University of Rhode Island, Narragansett, RI

Heat Shock (Stress) Response in Winter Flounder Renal Proximal Tubule Primary Cultures
M. Brown, R. Upender, L. Hightower, and J. L. Renfro
University of Connecticut, Storrs, CT

Delineation of Inshore Winter Flounder Stocks in Massachusetts—Preliminary Results Using Digital Image Analysis
J. A. Darde
National Marine Fisheries Service, Woods Hole, MA

Hepatic Cytochrome P-450E Induction, PCB Concentration, and Reproductive Parameters in Winter Flounder from Contaminated Environments
A. A. Elskus[1, 2], J. J. Stegeman[1], L. C. Susani[1], D. Black[3], R. J. Pruell[3], and S. J. Fluck[4]
[1]Woods Hole Oceanographic Institution, Woods Hole, MA, [2]Boston University Marine Program, Woods Hole, MA, [3]U. S. Environmental Protection Agency, Narragansett, RI, and [4]Science Applications International Corporation, Narragansett, RI

Mass-marking Juvenile Winter Flounder by Tetracycline Immersion
T. R. Gleason[1], T. G. Daniels[1], and R. Haas[2]
[1]Science Applications International Corporation, Narragansett, RI, and [2]University of Rhode Island, Kingston, RI

Genetic Analysis of Population Subdivision in Winter Flounder
K. A. Goddard[1] and J. R. Powell[2]
[1]American University, Washington, D.C. and [2]Yale University, New Haven, CT

Cytogenetic and Cytologic State and Mortality of Embryos of Winter Flounder, *Pseudoploeuronectes americanus,* from Long Island Sound and Boston Harbor
J. B. Hughes, D. M. Perry, and A. T. Hebert
National Marine Fisheries Service, Milford, CT

Growth and Survival of Winter Flounder Larvae in Mesocosms (Experimental Marine Ecosystems)
G. Klein-MacPhee, B. K. Sullivan, and A. Keller
University of Rhode Island, Narragansett, RI

Cytochrome P-450E Induction in the Winter Flounder by 3, 3'4, 4'-tetrachlorobiphenyl (Congener 77)
E. Monosson[1] and J. Stegeman[2]
[1]U. S. Environmental Protection Agency, Narragansett, RI, and [2]Woods Hole Oceanographic Institution, Woods Hole, MA

Serum Vitellogenin in Tumored and Nontumored Winter Flounder from the Boston Harbor Area
J. J. Pereira, J. Ziskowski, R. Mercaldo-Allen, and C. Kuropat
National Marine Fisheries Service, Milford, CT

Length-weight Relationship of Winter Flounder from Massachusetts Waters
D. B. Witherell, A. B. Howe, T. P. Currier, and S. J. Correia
Massachusetts Division of Marine Fisheries, Sandwich, MA

New Perspectives on Fin Erosion Disease on New Haven Harbor Winter Flounder from Prevalence, Physiological, and Radiographic Studies
J. Ziskowski, J. J. Pereira, R. Mercaldo-Allen, and C. Kuropat
National Marine Fisheries Service, Milford, CT

Abstracts
Oral Presentations

The 12-Mile Dumpsite Recovery Study: Preliminary Findings Relative to Winter Flounder

National Marine Fisheries Service, Northeast Fisheries Center
Sandy Hook Laboratory, Highlands, NJ 07732

Experimental Design of 12-Mile Dumpsite Recovery Study - An Overview

R. A. Pikanowski

The Environmental Processes Division of the Northeast Fisheries Center completed a 3-year study in September 1989, the primary purpose of which was to assess the effects of sewage on the ecology of the New York Bight Apex. Otter trawls were taken monthly, before and after the cessation of dumping in December 1987. The statistical method of "pseudoreplication in time" was used to determine the effect of sludge on winter flounder abundance as measured by systematic otter trawling.

Distribution, Relative Abundance, and Size-age Composition of Winter Flounder (*Pseudopleuronectes americanus*) Collected during the 12-Mile Dumpsite Recovery Study

S. J. Wilk, R. A. Pikanowski, A. L. Pacheco, D. G. McMillan, and L. Stehlik

Over 950 otter trawl tows were made during the 12-Mile Dumpsite Recovery study, which was conducted between July 1986 and September 1989. Summary time and space as well as environmentally related observations of distribution, relative abundance, and size-age composition are given for all winter flounder collected.

A Study of Winter Flounder (*Pseudopleuronectes americanus*) Movements in the New York Bight

B. A. Valdes

As part of the 12-Mile Dumpsite Recovery Study, tagging of winter flounder was initiated in July 1986 to determine the magnitude and extent of movements between the dumpsite area and the surrounding inshore areas. Through August 1989, 7,346 winter flounder (≥18 cm) were tagged at 22 offshore stations associated with the sewage sludge dumpsite and 14 inshore stations in the Sandy Hook Raritan-Lower Bay area. To date, 189 tags have been recovered, primarily by recreational fishermen (86.2%), with a total return rate of 2.6%. Based on the number of returns, several trends are suggested: (1) winter flounder within the study area exhibit generally accepted seasonal patterns of migration; (2) the Navesink-Shrewsbury River system supports a population of winter flounder that return yearly during the spawning season; and (3) there is intermixing between populations from New Jersey, the dumpsite, and points north and east, indicating that populations may not be as discrete as previously believed.

Incidence of Disease in Winter Flounder (*Pseudopleuronectes americanus*) Collected during the 12-Mile Dumpsite Recovery Study

L. Pacheco and J. Rugg

The occurrence and distribution of fin rot and other expressions of disease have been previously reported from the New York Bight Apex. Incidence of fin rot, as well as other observations of disease in winter flounder collected during the 12-Mile Dumpsite Recovery Study, was generally less than 2% and, thus, reflects little or no change over the last decade.

A 3-year Assessment of Reproductive Success in Winter Flounder, *Pseudopleuronectes americanus*

D. A. Nelson and J. B. Hughes

National Marine Fisheries Service
Northeast Fisheries Center
Milford Laboratory
Milford, CT 06460

The conditions of eggs, embryos, and larvae from 200 female winter flounder from six sites in Long Island Sound (LIS) and two in the Boston Harbor area were compared over 2 and 3 years, respectively. Fish were spawned and embryos cultured at the Milford Laboratory. Contaminants were measured in the spawned eggs of the fish and their livers. PCB levels were significantly higher in the livers of fish from the Boston stations, than in those from Shoreham and Milford stations in LIS (1987, 1988). Eggs of Boston flounder were smaller than those of LIS fish (1987-1989) despite the greater age and size of the former. Early-stage embryos of Hempstead flounder had a relatively high incidence of mitotic abnormalities in 2 out of 3 years (1986, 1987). Embryos from Boston fish (1987) had fewer mitotic abnormalities than those from all LIS stations. New Haven flounder embryos had the most abnormalities for 2 years of the study. New Haven late-stage embryos had a higher incidence of cell dedifferentiation/death than did those from any other site (1987, 1988). In 1987, the normal decrease in activity of the regulatory enzyme MDH was highest in maturing embryos of fish from nonurban Shoreham and lowest in those from the urban stations. During early development, their gross malformation was higher than that in early embryos from all other sites except Deer Island, located between Boston Bay and Boston Harbor (1987). Malformation in embryos from Deer Island fish was higher at all developmental stages than in those from Long Island, located along the southeast approach to Boston Harbor, or from any LIS site. Mortality of flounder embryos was highest during very early development. Mortality was greatest for embryos from New Haven fish and least for those from Shoreham fish (1986-1988), with intermediate levels for all other stations, including Boston (1987). New Haven consistently produced the poorest percent hatch and lowest percent viable hatch during this study (1986-1988). New Haven flounder produced small larvae all 3 years. Boston flounder produced the smallest larvae of all stations monitored in 1987. Shoreham (1987, 1988) and the two Boston Harbor stations (1987) had the smallest yolk-sac volumes, while New Haven and Hempstead had the largest yolk-volumes. A conservative estimate is that one-fifth of the fecundity of LIS winter flounder is wasted by early embryo-genesis, and three-fifths, by the time of hatching. The gradient in effects on winter flounder eggs/embryos in LIS reflects the urban-to-nonurban pollutant transition.

Movements and Exploitation of the Niantic River Stock of Winter Flounder

D. J. Danila

Northeast Utilities Service Company
Northeast Utilities Environmental Laboratory
Millstone Nuclear Power Station
PO Box 128, Waterford, CT 06385

Over 2,000 mostly adult winter flounder were marked with disc tags from December 1980 through March 1983 to determine their movements and exploitation by the sport and commercial fisheries. About 83% of the fish were tagged in winter and early spring on their spawning grounds in the Niantic River. Recaptures included 287 fish from sports fishermen, 99 from the commercial fishery, and 336 from sampling activities. About one-third of the recaptures made by fishermen occurred within 2 months of release and two-thirds within a year. The maximum time at liberty was 3.2 years. Females tended to move longer distances than males, as 31% of their returns were from outside of local New London County waters as compared to 18% of the males. Of fish moving longer distances, four out of five were recaptured in waters to the east of the Niantic River, with returns from as far as Nantucket Shoals, Cape Cod, and Georges Bank. These findings are generally similar to those from several other winter flounder tagging studies, which showed mostly upcoastal movements in spring and summer before a return to spawning grounds in fall and winter. The results suggest that a considerable fraction of adult winter flounder populations south of Cape Cod mix and become susceptible to a number of different regional fisheries. Furthermore, these fish are subjected to varying water quality and bottom conditions during the course of year as individuals move farther from their natal estuary and nearby coastal areas.

Growth Rates of Juvenile Winter Flounder in Different Estuarine Habitats: A Comparison of Habitat Quality

S. M. Sogard and K. W. Able

Rutgers University Marine Field Station
Tuckerton, NJ 08087

A comparison of growth rates was conducted in an attempt to assess the relative quality of different estuarine habitats in supporting juvenile winter flounder populations. Four sites, two in eelgrass beds and two in areas with beds of macroalgae (Ulva lactuca), were compared. In a series of six experiments in 1988 and 1989, juveniles (18- to 70-mm standard length) were held for 10 days in cages over vegetated and adjacent unvegetated substrates at each site. In 1988, 16 cages with 3 fish each were used in each experiment; in 1989 the design was expanded to 24 cages per experiment. Within a site, individual fish grew better on sand substrates than in vegetation, but growth varied markedly among sites, suggesting that physical location within the estuary may play an important role over and above that of habitat. Patterns of relative growth among sites were consistent between the two years of the study. Growth, in general, declined with increasing fish size, ranging from negative growth for larger fish (40-70 mm) in poor-quality habitats to over one millimeter per day for small fish (<30 mm) in optimal habitats. Concurrent density estimates obtained through regular throw trap sampling suggested that juveniles occur throughout the estuary, but have slightly higher densities at sites supporting faster growth. Otoliths of caged fish were examined to test the short-term correspondence of increment widths with actual somatic growth.

Tumorigenesis in Winter Flounder

M. J. Moore, R. M. Smolowitz, and J. J. Stegeman

Woods Hole Oceanographic Institution
Department of Biology, Woods Hole, MA 02543

A variety of cellular abnormalities and lesions have been described in adult winter flounder, Pseudopleuronectes americanus, from Boston Harbor, but the cellular orgins of these lesions have not been reported. To address this issue, winter flounder ranging in size from 10- to 450-mm total length were collected from Boston Harbor and adjacent cleaner coastal locations, and their livers were examined histopathologically. Normal histology was evident in all flounder from Georges Bank and in the majority of near-coastal fish, whereas in fish from Boston biliary proliferation, abnormal cellular vacuolation , abnormal infiltrative basophilic cells, and macrophage aggregation were all first seen in fish less than 100-mm total length. Incidences of these nonneoplastic lesions increased steadily with increasing size, most markedly after sexual maturation. Grossly visible lesions and neoplastic foci were first seen in adults greater than 300-mm length. Incidence of neoplastic and nonneoplastic lesions was comparable for both genders, although adenomas were rare in males. Neoplasia was only rarely seen in the absence of the above nonneoplastic changes. Comparison of lesion incidence between sites suggests Deer Island fish to be more severely affected than flounder from other areas of Boston Harbor. Maintenance of Deer Island fish in cleaner Woods Hole water for 5 months prior to examination showed no major reduction in lesion incidence for all lesions except that of basophilic infiltration, suggesting this to be an early reversible change, possibly the most sensitive morphological indicator of changes in environmental quality. In contrast, vacuolar change seemed to be irreversible. This change was first seen in isolated single cells, in multiple cells in hepatic tubular distribution, in aggregations in cholangial structure, and in large grossly visible foci. These cells first appeared predominantly in a preductular and ductular location. Further study of these nonneoplastic cell types is necessary to better understand liver neoplasia in winter flounder.

Organic Anion Transport by Flounder Renal Proximal Tubule in Culture: Inhibition by 2, 4-D and DDA*

M. Dawson[1, 2] and J. L. Renfro[2]

[1]*National Marine Fisheries Service*
Northeast Fisheries Center
Milford Laboratory
Milford, CT 06460

[2]*University of Connecticut*
Marine/Freshwater Biomedical Sciences Center
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We used primary monolayer cultures of winter flounder proximal tubules to measure active transport of p-aminohippuric acid (PAH), a model compound for the transport system that removes a variety of anionic xenobiotics as well as normal metabolites. The culture system allows the separate measurement of secretory and reabsorptive flux and the simultaneous monitoring of the integrity of the tissue by measuring electrical characteristics and sodium-dependent gluocose transport, as indicated by phloridzin-sensitive short-circuit current. Unidirectional fluxes were measured in Ussing chambers under short-circuited conditions at PAH concentrations of 10 and 100 µM. PAH fluxes were measured in the presence of the herbicide 2, 4-dichlorophenoxyacetic acid (2,4-D) at concentrations from 10-8 to 5 x 10-3 M. Concentrations of 2,4-D above 10-4 M inhibited transport at both PAH concentrations with a Ki of 0.7 mM. At 10 µM PAH, 2,4-D had a biphasic effect, stimulating PAH transport to 160 ± 18% of control at 10-5 M 2,4-D. The stimulatory effect was not apparent at a PAH concentration of 100 µM. The phloridzin-sensitive short-circuit current was unchanged by the presence of 2, 4-D, an indication that the reduction in PAH transport was an effect specific to the transport system and did not represent damage to the tissue.

The effect on PAH transport of 2,2-bis (p-chlorophenyl) acetic acid (DDA) was measured at DDA concentrations ranging from 10-6 M to 5 x 10-4 M. At 10-6 M, DDA had no effect on transport at either PAH concentration. Higher DDA concentrations inhibited PAH transport, with a Ki of 0.03 mM. The phloridzin-sensitive short-circuit current was unchanged at the lower DDA concentrations, indicating no damage to the tissue. At 0.5 mM DDA, the secretory flux was 16% of control, whereas reabsorptive flux was 400% of control; phloridzin-sensitive short-circuit current decreased, indicating some damage to tissue integrity. This suggests that, in addition to inhibition of transport, there is a nonspecific effect on tissue integrity at high DDA concentrations, an observation that is consistent with reports of uncoupling of oxidative phosphorylation by DDA

*Research supported by NIH/NEIHS Grant No. ES 03848 to J. L. Renfro.

Age Determination of Winter Flounder in Rhode Island Using Sectioned Otoliths

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Two hundred ninety-four Rhode Island winter flounder from Warwick Neck and Whale Rock, Narragansett Bay, and Quonochontaug Pond in Rhode Island were successfully aged with the use of sectioned otoliths. Ages are preliminary, pending validation. Validation is under way, using marginal increment analysis of biweekly samples of otoliths over a 1-year period.

Phosphate Transport in Winter Flounder Renal Proximal Tubule Primary Cultures*

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We have adapted primary cell culture techniques to winter flounder proximal tubule with the objectives of studying transepithelial transport in controlled electrochemical environments (Ussing chambers) and examining long-term effects of regulatory factors on those transport processes. These tissues can be maintained in a functionally differentiated state for up to 30 days on floating collagen gels. In Ussing chambers, the transepithelial electrical characteristics are identical to those of the intact, perfused tubule. In fishes, phosphate is regulated by filtration (if glomerular), reabsorption, and secretion; however, the mechanisms of reabsorption and secretion have not been previously studied. Under control culture conditions, the epithelium reabsorbed phosphate. Net phosphate secretion could be stimulated by raising extracellular phosphate concentration (>0.5 mM) or by activation of protein kinase C with diacylgylcerol, phorbol ester, or phospholipase C. Reabsorption could be stimulated with 10 μM forskolin, an activator of adenylate cyclase. Inhibition of Na, K-APase with 10-4 M ouabain inhibited both secretion and reabsorption.

*Research supported by NSF.

Impacts of Treated Municipal Wastewaters on Development and Growth of Winter Flounder

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Effluents from municipal wastewater treatment facilities supply >98% of point-source pollutants and 13% of total fresh water input to the Hudson-Raritan Estuary. We are studying the effects of chlorinated effluents on the sensitive early life stages of three species of fish common to this estuary. One source of effluents is a publicly owned treatment facility that receives about half of its input from industrial sources. Batch-to-batch variability in the chemistry of this effluent was reflected in biological impact. Embryos of the winter flounder (Pseudopleuronectes americanus) had skeletal defects and decreased hatch and larval growth. Growth of juveniles, as represented by a fin regeneration assay, was depressed at concentrations ≤10%. Effluent from a second treatment facility (with 25% industrial origin) had threshold effect at 20% concentration. In these assays, the flounder, were about as resistant as mummichog (Fundulus heteroclitus) and less sensitive than the striped bass (Morone saxatilis).

Analysis of the effluent indicated relatively low levels of heavy metals. A toxic batch had 8 times the total chlorocarbon level of an innocuous batch (estimated by total ECD response). The GC/ion chromatogram of the toxic batch had higher levels and greater diversity of pollutants.

Research was directed toward identifying the organic fraction with the greatest biological impact to investigate possible mitigation schemes. Results indicate that this fraction varies from batch to batch but that there is always a deleterious biological effect.

The utility of winter flounder embryonic development, hatching success, posthatch growth, and juvenile fin regeneration in identifying and quantifying an environmental hazard is evident.

Some Factors Affecting the Abundance and Growth of Larval Winter Flounder in the Vicinity of Niantic, Connecticut

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Larval winter flounder abundance and size distributions were examined in the Niantic River since 1983 and in Niantic Bay since 1976 to determine what factors might affect larval survival and growth. The annual abundance of yolk-sac larvae in the river was directly related to the estimated total egg production of the Niantic River spawning stock, which indicated that the proportion of eggs hatching was reasonably constant from year to year. A comparison of annual larval survival rates (calculated from changes in abundance) to total egg production suggested the presence of density-dependent mortality. Examination of length-frequency distribution in the river indicated that a majority of the larval mortality occurred between the 3- and 5-mm size classes, which is the size range when first feeding occurs. Yolk-sac larvae were collected primarily in the river, and the abundance of later developmental stages increased over time in the bay, apparently as larvae were flushed from their spawning grounds. The estimated dates of peak abundance in the bay appeared to be directly related to mean water temperature during March and April. The timing of larval peak abundance would be a function of rates of recruitment and loss (including mortality and juvenile metamorphosis). Water temperature could affect the rate of loss due to metamorphosis because estimated laboratory and field growth rates were found to be positively correlated to water temperatures.

Winter Flounder Young-of-the-Year Growth and Survival in Mesocosm and Field Ecosystems*

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A major objective of research on marine fish populations is to explain variations in recruitment in terms of underlying biological and environmental control mechanisms. A major methodological problem with this research is that sampling difficulties preclude field measurements of many critical processes, yet results of controlled experiments on these processes may not be relevant to field conditions. We are using a combination of controlled experiments, field observations, and integrative mathematical models to quantify the factors controlling the growth and survival of early life stages of winter flounder in the Niantic River.

As a first step, we modified an existing energetics-based model of the feeding, growth, and starvation of winter flounder larvae from spawning to metamorphosis. The dynamics of a cohort is quantified by simulating up to several thousand individual larvae. We calibrated the model to results of winter flounder larval growth and starvation experiments performed in laboratory aquaria and in the Marine Ecosystems Research Laboratory (MERL) mesocosms. The initial model adequately simulated the average growth rates of individual winter flounder larvae in all of the experiments but underestimated the variance in individual growth rates. Modification of the model to simulate inter-individual variability in innate growth capacity resulted in closer agreement with the data.

Having used the experimental data to constrain values of the energetics parameters, we simulated the abundance and size distribution of winter flounder larvae in the Niantic River from (1) estimates of the number of larvae spawned during each week, (2) estimates of the abundance of winter flounder prey and predators, and (3) daily temperatures. Results of these simulations are being used to identify needs for improved field observations and to design additional controlled experiments in the MERL.

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Predicting Temperature-dependent Developmental Rate and Survival in Hatchling Winter Flounder

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Temperature has long been known to have a profound and direct influence on embryonic developmental rate and to reduce survival to hatching at extreme temperatures. We see two shortcomings of previous analyses. Using data from laboratory and field populations of winter flounder from Newfoundland and published data from other studies, we found (1) that developmental rate was nonlinearly related to temperature, with departures most acute at extreme temperatures, and (2) that recognizing dispersion in the timing of life history events (e.g., hatching, starvation) improves predictions of the ages at these events in nature and potentially improves predictions of survival and recruitment. We compared models widely used in the fisheries literature for characterizing temperature-dependent developmental rate and recommend use of a modified version of the Arrhenius equation. Lastly, we apply event analysis to predict age at hatching and time until starvation under arbitrary temperature regimes.

Factors Contributing to Variability in Size and Viability of the Eggs and Larvae of Winter Flounder, *Pseudopleuronectes americanus,* Reared in the Laboratory

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Winter flounder, an important resource species in the northwest Atlantic, consists of local stocks that spawn in the different bays and estuaries along the coast from late winter through spring. A study of larvae produced by adults collected at selected sites in southern New England showed a relation between mean dry weight at hatch and survival for the first month. Examination of eggs and larvae spawned by females collected at a single location (Narragansett Bay, Rhode Island) over the spawning season showed that female length and spawning time explained 60% of the variability in egg dry weight. Egg size increased with female size and decreased through the spawning season. The smallest females produced small eggs with low fertility and viable hatch. Egg size, fertility, viable hatch, and survival were lowest among late spawners.

In a separate study, water temperature during the final stages of gamete maturation (up to 51 days prior to spawning) and water temperature during the embryonic period were found to have an effect on larval size and biochemical composition at first feeding. In many cases the effects of water temperature during these periods were nonadditive. RNA content at first feeding indicated that larvae produced by adults acclimated to low temperatures (2ºC) were better suited for growth at low temperatures, while larvae produced by adults acclimated to higher temperatures (7ºC) were better suited for growth at higher temperatures. At first feeding, larvae were larger and in better condition (high protein and RNA content) when incubated at lower temperatures.

Condition of Winter Flounder Larvae in Narragansett Bay as Measured by RNA/DNA Ratio

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Growth in length of laboratory-reared winter flounder larvae is characteristically high during yolk-sac absorption (first week) but relatively low during the period of first feeding (second and third weeks), after which high growth rate resumes. RNA/DNA ratio showed a minimum mean ratio during the period of first feeding (second and third weeks), corresponding to the period of slow growth. Yolk-sac larvae and larvae older than 3 weeks had relatively higher ratios. For any given age, morphologically more advanced larvae had a higher RNA/DNA ratio. Within each morphological stage the ratio tended to be highest at its first appearance, declining to a low level prior to the disappearance of the stage from the population. The RNA/DNA ratio of post-first feeding larvae was positively correlated with instantaneous growth coefficient irrespective of age. Field larvae from Narragansett Bay, collected in spring of 1988, show similar trends; the ratios were low in first feeding larvae but exhibited a near-linear increase with increasing larval length beyond the first feeding stage. Difference in RNA/DNA ratios of larvae of similar length from the upper and lower Bay are apparent and may be related to differences in food availability. The study indicates that the first feeding larvae are in a "critical" stage.

Trophic Transfer of PAH Metabolites into Winter Flounder*

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Polycyclic aromatic hydrocarbons (PAHs) are precarcinogens that accumulate in sediments in aquatic ecosystems. Consequently, their availability to benthic organisms, their metabolism, and their potential transfer through aquatic food chains to man is a matter of environmental health concern. Many benthic organisms can metabolize PAH; therefore, the bioavailability of both the parent compound and metabolites must be considered. The relative bioavailability and metabolic fate of dietary benzo[a]pyrene (BaP) and one of its primary metabolites, benzo[a]pyrene-7,8-dihydrodiol (7,8-Diol), and a mixture of BaP metabolites produced by the polychaete Nereis virens was investigated in the winter flounder, Pseudopleuronectes americanus, using 14C- and 3H-labeled compounds. Bile, intestine, liver, and muscle contained the highest percentage of the dose on a whole-body basis, which, in all cases, was extensively metabolized. Mixtures of metabolites produced by worms or pure 7,8-Diol were less bioavailable than the parent compound. Regardless of what form of BaP was presented in the diet, metabolites and bound residues were observed in the liver. Patterns of metabolite accumulation differed between fish fed the parent compound and fish fed 7,8-Diol or worm metabolites and between tissues analyzed. These experiments unequivocally demonstrate the potential for food-chain transfer of PAHs and their metabolites between aquatic species and indicate that, once absorbed, metabolites can be further modified by the prey organism.

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Winter Flounder Movement- A Historical Review and Current Information on Narragansett Bay Winter Flounder Populations

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Studies on the seasonal movements of winter flounder have been conducted since the 1940s. Although there are regional differences throughout the range of the species in the timing of movement, the direction of movement, and the extent of movement, there are many similarities. A summary of what is known about winter flounder along the Atlantic coast is presented.

Movement patterns of Narragansett Bay winter flounder populations are not well documented. Because of this and the need to develop a winter flounder management plan for Rhode Island stocks, the Rhode Island Division of Fish and Wildlife initiated a long-term study on Narragansett Bay populations.

Since 1985 more than 7,000 legal-sized winter flounder have been tagged and released in the upper Bay and Mt. Hope Bay. Data from over 750 tag recoveries have been returned by recreational and commercial fishermen. These have provided valuable information on path and distance of migration, rate of movement, homing tendencies, and exploitation of the tagged populations.

An analysis of the spatial and temporal distribution of the tag recoveries over the last 3 years shows remarkable similarities in the seasonal movement patterns both within the Bay and offshore. These data are presented, and the seasonal movement pattern of Narragansett Bay winter flounder is characterized.

The Eye Movement System of the Flatfish: A Model for Studying Adaptation

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Flatfish provide a natural model for the study of adaptive changes in the vestibulo-ocular reflex system. During metamorphosis their vestibular and oculomotor coordinate systems undergo a 90-degree relative displacement. As a result, during swimming movements, different types of compensatory eye movements are produced before and after metamorphosis by the same vestibular stimulation. We have now described the entire vestibulo-oculomotor circuitry in the adult winter flounder, Pseudopleuronectes americanus, utilizing extracellular and intracellular staining methods with horseradish peroxide. Furthermore, we largely characterized the system regarding its neurophysiology, transmitters, and ultrstructure. Our data indicate that second-order vestibulo-oculomotor neurons alone provide the adaptation to the postmetamorphic condition of the eye movement system: in the adult flatfish, neurons receiving horizontal semicircular canal input contact vertical eye muscle motoneuron pools on both sides of the brain via pathways that are absent in all other vertebrates studied.

The challenge for ongoing and future research lies in determining the factors that define flatfish at the level of the vestibular system and in describing the embryogenesis of the postmetamorphic eye movement circuitry.

Use of Radiopharmaceuticals for Evaluation of Boston Harbor Winter Flounder

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The pollution of Boston Harbor is well known, and pollution-related abnormalities of Boston Harbor winter flounder have been well documented. In the present study, winter flounder from Boston Harbor have been examined with four radiopharmaceuticals to determine their applicability for in vivo determinations of physiological/pathological alteration. The fish were anesthetized and injected by cardiac puncture with 99mTc-labeled RBCs, 99mTc-MDP, 201T1, or 111In human lgG; the distribution of specific agents was determined by scintigraphing and gamma well counting.

All of the agents distributed initially by blood flow. Gated blood pool imaging yielded an ejection fraction of 22%, consistent with the low-pressure, two-chambered heart. After 24-hours, the Tc-MDP localized primarily in bone, particularly in the scales and premaxillae. Uptake also occurred in an as-yet-unidentified lesion of the skeletal muscle in one animal. Thallium-201 is a potassium analog, which localized most intensely in myocardium and secondarily in kidney, liver, and spleen. At 20 hours, the lgG accumulated primarily in the liver, with lesser amounts in the spleen and kidney.

A limitation of standard pathology analyses makes it necessary to kill the subject, thus precluding follow-up studies of the progression and ultimate effects of specific conditions. The present results indicate that standard radiopharmaceutical methods can be applied to winter flounder and that consistent physiological data can be derived. Further work will be directed toward habitat-specific comparisons of function, serial diagnoses of lesions, and the potential application of these techniques to impact assessment and monitoring.

Habitat Utilization by Winter (*Pseudopleuronectes americanus*) and Smooth (*Liopsetta putnami*) Flounders in Great Bay Estuary, New Hampshire

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Winter and smooth flounders are sympatric over much of their ranges and are morphologically very similar. However, little is known of their interactions within estuaries, which are important nursery areas for both species. This study was initiated to determine the amount of overlap in the distribution of these two species in Great Bay estuary. Nine habitats differing in salinity, depth, or bottom substrate were sampled monthly by 16-ft otter trawls. The habitats sampled included tidal and subtidal mud flats; eelgrass; oligohaline; riverine; oligomesohaline riverine; shallow (<15-ft), mid-depth, (15- to 25-ft), and deep (>25-ft) estuarine channels; and shallow sand bottom. Shallow stations could be sampled only at high tide, but deeper stations (estuarine channels and riverine) were sampled at low, middle, and high tides. Relatively few flounders of either species were caught in eelgrass, sand bottom, deep estuarine channel, or mud flat habitats. Smooth flounders were most abundant in the low-salinity riverine habitats, suggesting they may fill an ecological role occupied by hogchokers (Trinectes maculatus) south of Cape Cod. Winter flounder were most abundant in the mid-depth estuarine channels. They appear to be more restricted in their habitat requirements. However, there was a considerable amount of overlap in the area utilized by these two species, providing a possibility of competitive interactions.

Abstracts

Poster Presentations

Development of Potential Toxicity Indices in Isolated Winter Flounder Hepatocytes

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In vitro test systems are being developed with isolated hepatocytes from winter flounder to study modes of action of toxicants and the role of metabolism in mediating toxicity. Methods were developed for the isolation and culture of hepatocytes. The determination of optimal cell culture conditions was assessed by cell viability (trypan blue exclusion), protein synthesis (incorporation of ^{3}H-leucine), and intracellular potassium (inductively coupled plasma emission spectroscopy). The effects of cadmium on these parameters were also investigated. Flounder hepatocytes were isolated by a two-stage collagenase perfusion that resulted in a yield of 6×10^8 to 30×10^8 cells per liver with an average viability of 91%. Initial experiments were performed to determine optimal cell culture conditions. Four media (RPMI 1640, Suspension Minimal Essential Media, Medium 199, and L15) were evaluated in suspension culture. Thirty million cells were incubated in 10 mL of media with 10 µCi ^{3}H-leucine. Highest cell viability and protein synthesis levels were obtained with RPMI 1640 media. This medium was used for all subsequent experiments. Isolated winter flounder hepatocytes were exposed to 10-1750 µM cadmium. Protein synthesis was found to be a much more sensitive indicator of toxicity than trypan blue exclusion.

The Effect of Hypoxia on the Growth of Young-of-the-Year Winter Flounder

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The effect of constant and diurnally occurring hypoxia on the growth of young-of-the-year winter flounder, *Pseudopleuronectes americanus*, was examined under controlled laboratory conditions. Two groups of flounder, groups I and II, were exposed for 11 and 10 weeks, respectively, to constant dissolved oxygen (DO) concentrations of 6.7 and 2.2 mg/L and to a diel fluctuation of DO ranging from 2.5 to 6.4 mg/L. Growth was significantly reduced ($P<0.001$) by exposure to both types of hypoxic conditions with fish held at 2.2 mg/L exhibiting the greatest impairment. Increases in length averaged 3.56 mm/week at 6.7 mg/L, 150 mm/week at 2.2 mg/L, and 29 mm/week with fluctuating DO. At the end of the exposure period, all fish were held at DO concentrations averaging 7.2 mg/L for 5 weeks. Growth rates during this period indicate that impairment of growth is transient.

A Gross and Histological Atlas of Winter Flounder Larvae: Progress to Date

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Presently we are preparing an atlas on the gross and histological morphology of winter flounder larvae reared under controlled conditions. Specimens for this study were taken from the time of hatching through the first 4 weeks of life. The atlas is modeled after Groman's 1982 work on the histology of striped bass (*Morone saxatilis*) and will include a substantive text describing the salient features included in the illustrations. In addition, histopathological information on a subset of larvae starved continuously from the time of hatching and sampled simultaneously with fed animals will be included. It is our impression that larval fish biologists interested in the ecology, bioenergetics, and ecotoxicology of this species or other pleuronectids will find this reference work of value.

Heat Shock (Stress) Response in Winter Flounder Renal Proximal Tubule Primary Cultures*

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We have begun characterizing the stress response of winter flounder renal epithelium maintained in primary culture at 20ºC. It was found that stress proteins of three size categories, 90kD, 70kD, and 30kD, were induced in these cultures after exposure to heat for 1 hour. Not only were these proteins synthesized immediately after stress, but they accumulated to high levels 8 hours later. Each class of protein was observed to have its own optimum temperature for induction, and protein synthesis was inhibited by temperatures of 31ºC or higher. Some transport and electrical characteristics of the cultures were also measured after heat stress. These parameters were more resistant to elevated temperatures. The response of tissue to disaggregation, plating, and reorganization was also investigated. The level of the small heat shock protein showed a peak at day 4 after plating, but by day 10 it had returned to a control level. Also at day 10, the cultures were determined to be functional as defined by their electrical and transport properties. The possibility that the small heat shock protein is involved in repair/recovery of the renal epithelium in culture is under investigation.

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*Research sponsored by the NIEHS Marine/Freshwater Biomedical Sciences Center.

Delineation of Inshore Winter Flounder Stocks in Massachusetts: Preliminary Results Using Digital Image Analysis

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Digital image analysis techniques were applied to winter flounder scale and otolith samples to assess the feasibility of this methodology in conducting stock identification studies for the species. Circuli spacing and patterns and scale shape were examined for 200 samples collected during Northeast Fisheries Center research activities from areas north and south of Cape Cod. Additionally, otoliths from 329 juvenile winter flounder seine-captured at four Cape Cod sites during 1986 and 1987 were evaluated with respect to growth patterns quantitatively identified in a previous study. Preliminary results suggested that, unlike scales of many anadromous species, winter flounder scales may be only marginally useful structures for stock separation. Analysis of otolith microstructure suggested potential for the methodology as a means of classifying winter flounder according to systems of origin.

Hepatic Cytochrome P-450E Induction, PCB Concentration, and Reproductive Parameters in Winter Flounder from Contaminated Environments

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Reproductively mature winter flounder (Pseudopleuronectes americanus) were collected from three northeastern U.S. sites with different degrees of ploychlorinated biphenyl (PCB) and polynuclear aromatic hydrocarbon (PAH) contamination. Liver PCB concentrations (measured by capillary EC-GC) in fish collected in 1987 and 1988 ranged from 7.4 to 191 µg/g dry wt at New Bedford Harbor (NBH), from 3.9 to 17.7 µg/g at Gaspee Point, and from 1.6 to 15.1 µg/g at Fox Island. Levels of ethoxyresorfin-O-deethylase (EROD) activity were similar in fish of the same reproductive status from the three sites; however, immunoquantitated P-450E homologue (the EROD catalyst) content was significantly higher in NBH fish. This suggests that P-450E catalytic activity is being suppressed in the livers of the NBH animals. Recent studies in our laboratory indicate that competitive inhibition of P-450E catalytic activity by specific PCB congeners is one likely mechanism of this suppression. At all sites hepatic EROD activity and P-450E content were significantly lower in gravid females (EROD, 0.10 to 0.69 units per nanomole P-450; P-450E, 8.4 to 19% of spectral P-450) than in spent females (EROD, 1.94 to 3.49; P-450E, 48 to 109%) and in ripe males (EROD, 1.86 to 3.41; P-450E, 48 to 84%). This finding is consistent with a hormonal effect on P-450E expression, and thus on EROD activity, in gravid females. The data indicate a complex relationship between levels of EROD activity, or P-450E, and tissue PCB concentrations in highly contaminated fish. How these variables are linked to altered endocrine or gonadal function is not yet known.

Mass-Marking Juvenile Winter Flounder by Tetracycline Immersion

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Twenty-one juvenile winter flounder (*Pseudopleuronectes americanus*) were immersed in a solution of tetracycline hydrochloride (500 mg/L) and monovalent salts nearly isotonic to seawater (28 ppt) for 9 hours. Twenty-one days after immersion, marked fish could be distinguished from control fish under ultraviolet light; the craniums of live winter flounder exposed to tetracycline fluoresced. Fluorescence was also detectable at the edges of the astericus and sagittal otoliths. No growth increments were observed beyond the tetracycline mark. Ongoing experiments are attempting to determine the optimum immersion solution and tetracycline dosage for most effectively mass-marking young winter flounder while minimizing stress associated with immersion.

Genetic Analysis of Population Subdivision in Winter Flounder

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Winter flounder live in localized populations from Nova Scotia to the Chesapeake Bay, breeding in estuaries during the late winter and early spring. Tagging studies indicate that they migrate short distances offshore to feed outside the breeding season, and most return to the same estuary to spawn every year. To determine if these behaviors have led to the development of genetic differences between populations breeding in separate estuaries, the mitochondrial DNA (mtDNA) genotypes of individuals from ten populations were compared.

Winter flounder were collected in 1988 on the breeding grounds from six populations in Connecticut and from one population each in Rhode Island, New Jersey and Long Island. Juvenile fish were collected after the breeding season in New Brunswick, Canada. Over one-half of the individuals had unique mtDNA genotypes identified by using four or more endonucleases. Therefore, the geographical distribution of more basic mtDNA genotypes, determined by using three or fewer restriction endonucleases, was examined. All genotypes determined by three or fewer endonucleases were found in fish over the entire study area. Several methods of statistical analyses (e.g., Fst, log-likelihood G-test) indicate that populations do differ in the proportions of genotypes represented, suggesting that some population restructuring has occurred since the glaciers receded and winter flounder invaded the North Atlantic.

Cytogenetic and Cytologic State and Mortality of Embryos of Winter Flounder, *Pseudopleuronectes americanus*, from Long Island Sound and Boston Harbor

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A study was conducted during 1986-1988 to compare the early reproductive success of 200 winter flounder, *Pseudopleuronectes americanus*, females collected from six sites in Long Island Sound and two sites in Boston Harbor. Effort was focused on examination of all developmental stages for mortality and on stage III (blastula) and stage VI (tail-bud) embryos for cytogenetic and cytological condition. Cytogenetic/cytological abnormalities observed include abnormal cell differentiation, chromosome breaks and bridges, depressed mitotic rate, abnormal chromosome numbers, and spindle defects. Mean station percent of abnormal mitoses at the blastula stage ranged from 0.5% at Deer Island, located between Boston Bay and Boston Harbor, to 15.4% at Hempstead. Mean station percent of tail-bud embryos with depressed mitotic rate ranged from 0% at Milford and New Haven to 36.5% at Hempstead. Abnormal cell differentiation ranged from a mean station percent of 1.4% at Shoreham to 11.1% at Milford. In all 3 years of the study, at both early and late development, mortality was three times greater for embryos from New Haven than for those from Shoreham. All flounder with more than 75% embryo mortality came from either New Haven, Hempstead or Boston Harbor. Embryo mortality ranged from 0% to 100% for individual fish. Overall, data indicate that New Haven is the most seriously stressed site in this study, while embryos from other sites, notably Hempstead and both Boston Harbor stations, show subtle indications of strain.

Growth and Survival of Winter Flounder Larvae in Mesocosms (Experimental Marine Ecosystems)

G. Klein-MacPhee, B. K. Sullivan, and A. Keller

University of Rhode Island
Graduate School of Oceanography
Narragansett, RI 02882-1197

Newly hatched winter flounder larvae were reared in 13-m^3 mesocosms to determine growth and survival at two levels of nutrient enrichment, and with and without benthic sediments.

Larval survival for 2 weeks was 6 and 20% in tanks containing sediments and 60 and 74% in tanks without sediments. Survival at the end of 4 weeks at two levels of nutrient enrichment showed no significant differences in growth of the larvae. Survival was not significantly different in three of the four tanks (47, 47, and 46%). This is the highest recorded survival for this species under control conditions. One tank had only 16% survival because of oxygen supersaturation. Mortality coefficients of winter flounder in nutrient-enriched tanks in experiment 2 compared favorably with those in laboratory experiments in which high food levels were provided. Growth was comparable to that of winter flounder reared in the laboratory at high food concentrations.

The value of these mesocosms as a research tool in larval fish studies is discussed.

Cytochrome P-450E Induction in the Winter Flounder by 3, 3'4, 4'-tetrachlorobiphenyl (Congener 77)*

E. Monosson[1] and J. Stegeman[2]

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Narragansett, RI 02882

[2]Woods Hole Oceanographic Institution
Woods Hole, MA 02543

Induction of cytochrome-P-450E in winter flounder subsequent to treatment with congener 77 was evaluated in fish from two sites: Georges Bank, a clean site, and Narrow River (Narragansett, RI). Untreated fish from these two sites showed markedly different levels of P-450E with high levels present in Narrow River fish, indicating significant prior exposure to inducers. Total hepatic cytochrome P-450, ethoxyresorufin-O-deethylase (EROD) activity, and P-450E were increased with increasing dosages of congener 77, from 0.1 to 10.0 mg/kg in fish from Georges Bank. Turnover number (activity per nanomole of P-450E) was decreased. Winter flounder from Narrow River showed a different response to congener 77 than did Georges Bank fish. Total P-450 content tended to decrease rather than increase, and EROD was significantly decreased in Narrow River flounder. P-450E content tended to increase at the higher doses of 77, but this increase was not statistically significant. The turnover numbers (EROD per nanomole of P-450E) in the Narrow River fish were lower than those in Georges Bank fish and showed a tendency to decrease even with further congener 77 treatment. These data indicate that there are processes of induction and inhibition of cytochrome P-450E by congener 77 similar to results reported previously for scup (Gooch et al. 1989 [Toxicol. Appl. Pharmacol 98:422]).The data also indicate that previous induction of cytochrome P-450E can influence the responses to additional exposure to chlorobiphenyl inducers. The mechanism and significance of inhibition or suppression of P-450E activity by polychlorinated biphenyls are not understood and warrant further investigation. In any case these results have important implications for use of P-450E measurements in biomonitoring.

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*Research supported by a National Research Council Postdoctoral Associateship and EPA agreement CR813155.

Serum Vitellogenin in Tumored and Untumored Winter Flounder from the Boston Harbor Area

J. J. Pereira, J. Ziskowski, R. Mercaldo-Allen, and C. Kuropat

National Marine Fisheries Service
Northeast Fisheries Science Center
Milford Laboratory
Milford, CT 06460

In March 1988, winter flounder were collected in Quincy Bay, Massachusetts, near Long Island. Blood samples were taken, and the gonadal development and liver condition of each fish were noted. The blood was refrigerated, allowed to clot overnight, and centrifuged immediately the following day. Serum was collected with a Pasteur pipette and frozen at –40ºC for later analysis.

Measurement of alkali-labile phosphate (ALP) in serum was used as an indicator of the yolk-precurser protein vitellogenin. Statistical analysis of ALP values showed them to be significantly lower ($P \leq 0.05$) in fish with obvious gross liver lesions than in those without such lesions. Lowered serum vitellogenin may result in higher rates of oocyte atresia and reduced fecundity.

Length-Weight Relationship of Winter Flounder from Massachusetts Waters

D. B. Witherell, A. B. Howe, T. P. Currier, and S. J. Correia

Massachusetts Division of Marine Fisheries
18 Route 6A
Sandwich, MA 02563

The length-weight relationship of winter flounder from southern New England and Georges Bank has been described in the literature, but these observations were based on measurements of iced fish. To get more precise length-weight information, we measured 662 live winter flounder from Massachusetts's waters during the May DMF bottom trawl survey. Low variability of weight at length was observed for males (r^2= 0.98) and females (r^2= 0.99). The length-weight constants calculated from our data (males: b= 3.001, log c=-4.912; females: b= 3.154, log c= -5.263) were higher than the spring values reported in the literature, therefore, calculations of weight from length using published length-weight constants may be underestimated.

New Perspectives on Fin Erosion Disease on New Haven Harbor Fish from Prevalence, Physiological, and Radiographic Studies

J. Ziskowski, J. J. Pereira, R. Mercaldo-Allen, and C. Kuropat

National Marine Fisheries Service
Northeast Fisheries Science Center
Milford Laboratory
Milford, CT 06460

Trawling operations in New Haven Harbor in spring 1989 revealed 38% fin erosion prevalence among ≥ 2-year-old female winter flounder. This represents a sevenfold increase of the prevalence of this disease as compared to that among yearling fish. Only 15% of ≥3-year-old female flounder have fin erosion, indicating possible mortality of affected fish in this polluted estuary. Female flounder may be more affected by fin erosion since they represented more than 90% of diseased catches in spring 1987 and 1988 and more than 70% of diseased fish in 1989. Also, only 24% of ≥ 2-year-old male flounder captured in spring 1989 were fin eroded.

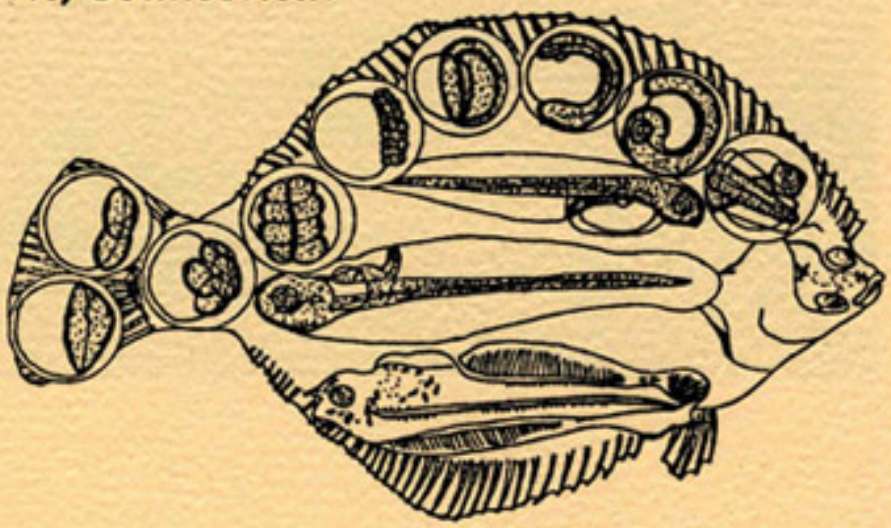
A fitness index based on body thickness vs standard length was applied to the spring 1989 catch; emaciation of diseased flounder of both sexes was confirmed.

The bent fin ray condition is closely associated with fin erosion disease; x-radiography demonstrated a weakened and "bent" fin ray structure adjacent to active lesions.

Analysis of blood drawn in 1988 from nine ≥3- and ≥4-year-old fin-eroded female flounder paired with appropriate unaffected fish from the same catch indicated lower hematocrits ($P < 0.05$) in the diseased fish. There is an indication that serum osmolalities and calcium levels are elevated in fin-eroded fish. However, a larger sample size is necessary to confirm these preliminary findings. Total protein, phosphate, and bilirubin levels are the same in both groups of flounder.

Winter Flounder Biology Workshop

December 3-4, 1991
Mystic, Connecticut



Program and Abstracts

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Winter Flounder Biology Workshop
December 3-4, 1991, Mystic, Connecticut

by Conference Steering Committee: Anthony Calabrese (Chair)[1], Allan Beck[2], Jay Burnett[3], Donald Danila[4], Arnold Howe[5], Penelope Howell[6], Ambrose Jearld[3], Chris Powell[7], and Anne Studholme[8]

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Third in a series of Flatfish Biology Conferences

U.S. DEPARTMENT OF COMMERCE
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Winter Flounder Biology Workshop

December 3-4, 1991, Ramada Inn, Mystic, Connecticut

Oral Presentations

Tuesday, December 3rd

8:00 a.m. **Registration/Coffee-Ramada Inn**

9:00 a.m. Welcome and Introduction
Robert A. Murchelano, Chief, Environmental Processes Division
National Marine Fisheries Service
Woods Hole, MA

Anthony Calabrese, Conference Chair
National Marine Fisheries Service
Milford, CT

Session I

Anne Studholme, Chair
National Marine Fisheries Service
Sandy Hook, NJ

9:30 a.m. Habitat Issues in the Management of Inshore Stocks of Winter Flounder
P. Howell
Connecticut Department of Environmental Protection, Waterford, CT

10:00 a.m. Vertical Distribution of Yolk-sac Winter Flounder Larvae in the Niantic River, CT
J. D. Miller
Northeast Utilities Environmental Laboratory, Waterford, CT

10:30 a.m. **Coffee Break**

Session II

Allan Beck, Chair
Environmental Advantage Group
Prudence Island, RI

10:45 a.m. Effects of the Bloom of the Diatom *Stephanopyxis turris* in Narragansett Bay in 1988 on Winter Flounder Larvae
G. Klein-MacPhee and E. Hjorleifsson
University of Rhode Island, Narragansett, RI

11:15 a.m. Post-metamorphic Success in Winter Flounder: Identifying the Selective Agents
R. C. Chambers, D. F. Bertram, and W. C. Leggett
McGill University, Montreal, Quebec, Canada

11:45 a.m. Predation on Juvenile Winter Flounder: Effects of Prey Size and Predator Density
D. A. Witting and K. W. Able
Rutgers University Marine Field Station, Tuckerton, NJ

12:15 p.m. **Hosted Lunch**

Session III

Jay Burnett, Chair
National Marine Fisheries Service
Woods Hole, MA

1:30 p.m. Habitat Relationships in YOY Winter Flounder
L. A. Deegan[1] and S. E. Saucerman[2]
[1]Marine Biological Laboratory, Woods Hole, MA and [2]University of Massachusetts, Amherst, MA

2:00 p.m. Estimation of Winter Flounder Spawning Stock Abundance in the Niantic River
D. J. Danila
Northeast Utilities Environmental Laboratory, Waterford, CT

2:30 p.m. Growth and Maturation of Winter Flounder in Massachusetts Waters
D. Witherell[1] and J. Burnett[2]
[1]Massachusetts Division of Marine Fisheries, Sandwich, MA and [2]National Marine Fisheries Service, Woods Hole, MA

3:00 p.m. **Coffee Break and Poster and Exhibit Set-up**

Session IV

Don Danila
Northeast Utilities Environmental Laboratory
Waterford, CT

3:30 p.m. The Reproductive Cycle in Winter Flounder off Newfoundland
M. Burton
Memorial University of Newfoundland, St. John's, Newfoundland, Canada

4:00 p.m. Exploratory Stimulation of Some Environmental and Biotic Factors Affecting YOY Winter Flounder Growth and Survival
K. Rose[1], C. Chambers[2], Tyler[1], G. Klein-MacPhee[3], and D. Danila[4]
[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]McGill University, Montreal, Quebec, Canada, [3]University of Rhode Island, Narragansett, RI and [4]Northeast Utilities Environmental Laboratory, Waterford, CT

4:30 p.m. Winter Flounder Trophodynamics in the Stressed New York Bight Apex
F. Steimle
National Marine Fisheries Service, Sandy Hook, NJ

5:00 p.m. **Poster and Exhibit Set-up**

5:30 p.m. **Hosted Mixer and Poster and Exhibit Session**

Wednesday, December 4th

8:00 a.m. **Registration/Coffee**

Session V

Arnold Howe, Chair
Massachusetts Division of Marine Fisheries
Sandwich, MA

8:30 a.m. Effects of Environmental Salinity and Thyroid Axis Manipulation on Survival and Growth in Juvenile Winter Flounder
T. A. Whitesel, K. W. Able, and M. L. Keefe
Rutgers University Marine Field Station, Tuckerton, NJ

9:00 a.m. Questions on Variability in MFO Measurements from Wild Winter Flounder Stocks in Nova Scotia Waters
J. H. Vandermeulen, D. Mossman and V. Vignier
Bedford Institute of Oceanography, Department of Fisheries and Oceans, Dartmouth, Nova Scotia, Canada

9:30 a.m. Effects of Organochlorine Pesticides on Active Organic Anion Secretion by Primary Cultures of Winter Flounder Proximal Tubules
M. A. Dawson[1, 2] and J. L. Renfro[2]
[1]National Marine Fisheries Service, Milford, CT and [2]University of Connecticut, Storrs, CT

10:00 a.m. **Coffee Break**

Session VI

Chris Powell, Chair
Rhode Island Division of Fish and Wildlife
Kingston, RI

10:30 a.m. Antibody Levels Against Bacterial Pathogens are Increased in Winter Flounder from Polluted Sites
R. A. Robohm and D. Kapareiko
National Marine Fisheries Service, Milford, CT

11:00 a.m. The Cytopathology of Fin Erosion Disease in Winter Flounder
J. E. Bodammer
URI/ NOAA Cooperative Marine Education and Research Program, Kingston, RI

11:30 a.m. Hydropic Vacuolation in the Liver of Winter Flounder
M. J. Moore and J. J. Stegeman
Woods Hole Oceanographic Institution, Woods Hole, MA

12:00 p.m. Risk of Disease in Winter Flounder of Northeast Estuaries
S. A. MacLean and C. Meise-Munns
National Marine Fisheries Service, Narragansett, RI

Poster and Exhibit Session
Tuesday, December 3, 5:30 p.m.

Posters

Cloning of Two P-glycoprotein Genes in Winter Flounder (*Pseudopleuronectes americanus*)
K. M. Chann[1], P. L. Davies[1], L. Veinot[2], and V. Ling[2]
[1]Queen's University, Kingston, Ontario, Canada, and [2]The Princess Margaret Hospital and University of Toronto, Toronto, Canada

Development of a Population Model for Anthropogenic Effects Upon Reproduction, Growth and Survival of Winter Flounder in Long Island Sound
Staff
National Marine Fisheries Service, Northeast Fisheries Science Center

Numerical Response of Winter Flounder to Herring Spawn in the Southern Gulf of St. Lawrence
R. Morin[1], R. Tallman[2] and D. K. Cairns[1]
[1]Gulf Fisheries Centre, Department of Fisheries and Oceans, Moncton, New Brunswick, Canada and [2]Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba, Canada

Exhibits

Ultrasonic tags
Image analysis
Beam trawls

Abstracts
Oral Presentations

Habitat Issues in the Management of Inshore Stocks of Winter Flounder

P. Howell

Department of Environmental Protection
Marine Fisheries Office
PO Box 248, Waterford, CT 06385

Habitat issues usually present an intractable problem for fisheries managers. Although all management plans recognize that productive habitat is needed, and all losses are undesirable, weighing the importance of losses due to poor environmental conditions against losses due to fishing is extremely difficult. Analysis of flounder life history shows that under present levels of fishing mortality, an increase or decrease of young-of-the-year mortality is roughly equivalent to the same relative change in fishing mortality in terms of its effect on lifetime egg production. For this species, conditions of high adult fishing mortality may make early life stage losses more critical.

Review of the winter flounder literature identified three anthropogenic mortality factors exerting long-term deleterious effects on winter flounder habitat quality: toxic contamination of nearshore waters, habitat loss and alteration, and power plant entrainment and impingement. Specific effects are quantified as much as possible, but further study incorporating these mortality factors into fishery yield models is needed.

Vertical Distribution of Yolk-sac Winter Flounder Larvae in the Niantic River, CT

J. D. Miller

Northeast Utilities Environmental Laboratory
Millstone Nuclear Power Station
PO Box 128, Waterford, CT 06385

Yolk-sac winter flounder larvae were sampled at discrete depths in the Niantic River using a specially designed pump sampler. The sampler design allowed the collection of larvae prior to their passage through the pump, which would destroy yolk-sac winter flounder larvae. The purpose of the study was to determine if yolk-sac larvae congregated at the sediment-water interface. This behavior would retard their flushing from the river.

Twelve sets of samples were collected at three stations during February and March of 1991. Each set of samples consisted of collections at the surface, mid-depth, near bottom (approximately 0.3 m above the bottom), and at the sediment-water interface. Generally, the highest larval densities were found at near-bottom (geometric mean=214 per 500 m^3) and at mid-depth (324 per 500 m^3), with the lowest densities at the sediment-water interface (95 per 500 m^3) and at the surface (23 per 500 m^3). Densities at the sediment-water interface were significantly ($a < 0.05$) lower than at near-bottom and mid-depth. It did not appear, therefore, that yolk-sac larvae utilized vertical positioning in the water column as a retention mechanism.

Effects of a Bloom of the Diatom *Stephanopyxis turris* in Narragansett Bay in 1988 on Winter Flounder Larvae

G. Klein-MacPhee and E. Hijorleifsson

University of Rhode Island
Graduate School of Oceanography
Narragansett, RI 02882

A phytoplankton bloom of the diatom Stephanopyxis turris occurred in April 1988 in Narragansett Bay, Rhode Island. This diatom is part of the natural plankton community of Narragansett Bay, but normally occurs in low numbers and rarely dominates the spring plankton bloom. Winter flounder larvae and zooplankton collected April 1 and 4 in the Providence River were relatively immobile and appeared anesthetized in the sorting trays compared to fish collected at Greenwich Bay and Wickford Harbor on the same dates. The bloom traveled down the bay in the succeeding week and occurred in the Marine Ecosystems Laboratory mesocosms where winter flounder experiments were in progress.

The bloom caused a depression in the numbers of winter flounder larvae collected in the Providence River and a decrease in the medium length of each stage on two sampling dates after peak bloom. Effects on feeding habits of winter flounder larvae in the field and in the MERL mesocosms are discussed.

Post-metamorphic Success in Winter Flounder: Identifying the Selective Agents

R. C. Chambers, D. F. Bertram, and W. C. Leggett

McGill University, Department of Biology
1205 Avenue Dr. Penfield
Montreal, Quebec, Canada H3A 1B1

The age, size, and date at which winter flounder metamorphose from the larval to juvenile stage are known to vary. Evaluating the consequences of this variation is the next significant challenge. The approach employed here uses data from laboratory rearings as estimates of expected post-metamorphic growth dynamics, and applies principles appropriate for the analysis of longitudinal data. Based on simulations, we demonstrate expected size distributions of young-of-the-year juvenile flounder from two scenarios of size-selective mortality: 1) size-specific mortality operating on recently metamorphosed juveniles, and 2) size-specific mortality operating via winterkill. We detail implications of these two scenarios and suggest field tests for discrimination between these mechanisms.

Predation on Juvenile Winter Flounder: Effects of Prey Size and Predator Density

D. A. Witting and K. W. Able

Rutgers University, Marine Field Station
Institute for Marine and Coastal Sciences
PO Box 278, Tuckerton, NJ 08087

Preliminary laboratory and field observations have suggested that the sand shrimp (*Crangon septemspinosa*) may be an important predator of juvenile winter flounder (*Pleuronectes americanus*). Two laboratory experiments were conducted to investigate this predator-prey relationship further. The first experiment tested the effect of winter flounder stage/size on rate of predation by similar sized shrimp (45-55 mm TL). Flounder stages ranged from pelagic larvae (eyes symmetrical, 5-8 mm SL), to 60 mm (SL) juveniles. Experiments were conducted in replicate cylinders (0.2 m^2 and 51 cm in depth). The highest predation was observed on winter flounder that were at pre-eye and post-eye migration stages, with lower levels of predation observed during eye migration and on larger juveniles. These experiments suggest a complex interaction between winter flounder size, stage, settlement behavior, and susceptibility to predation by sand shrimp.

The second experiment tested the effect of shrimp density on predation rate. Six predator densities (0-36 m^2) were tested in pools (1 m^2 and 16 cm in depth). Winter flounder size and density was held constant (11-13 mm and 5 m^2, respectively). When predator densities were lower than 9 m^{-2}, flounder mortality was low (0-20%); however, in treatments where shrimp densities were 9 m^{-2} or greater, flounder mortality increased to nearly 100%. These results demonstrate that predator density has a pronounced effect on survival of juvenile winter flounder, therefore, inter- and intra- habitat variation in predator density could play an important role in determining settlement and survival of juvenile winter flounder in the field. Together these laboratory experiments substantiate the significance of predator–prey interactions to survival of young-of-the-year winter flounder. Further field studies are underway to define the extent and role of these interactions in winter flounder population dynamics.

Habitat Relationships in YOY Winter Flounder

L. A. Deegan[1] and S. E. Saucerman[2]

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Department of Forestry and Wildlife Management
Amherst, MA 01003

Lateral and cross-channel exchange of young-of-the-year winter flounder were examined over a 40-day period in June and July, 1988, on Eel Pond, Massachusetts. Flounder were injected subcutaneously with two colors of acrylic paint and released at two locations on opposite sides of a channel. Recapture occurred one and three weeks after the last release date. Of the 275 recaptured fish, 98% were within 100 m of the release site, two (0.73%) were found on opposite sides of the channel, and three (1.09%) were found more than 200 m from the release site. Both release sites were near eelgrass beds, and the largest number of fish captured for marking were captured in the eelgrass areas. The highest percentage of recaptures were at the eelgrass beds where they had been initially captured. These results indicate that lateral and cross-channel movements of Y-O-Y winter flounder are limited in the summer months, and suggests that eelgrass plays an important role as nursery habitat for this species.

Twelve sites were studied in 1987 and 1988 at Waquoit Bay, Massachusetts, to evaluate age 0 winter flounder nursery habitats. The sites were classified as sand, silt or mud habitats, and as high, medium or low sediment organic content habitats. Significant differences were found in abundance, growth and condition of juvenile winter flounder at different sediment textures and levels of organic content. Abundance of juvenile winter flounder was highest in mud habitats with high organic content, and growth was lowest in the same habitat type. Condition of age 0 winter flounder was highest in high organic content habitats. These results illustrate the relative importance of different habitat types to juvenile winter flounder, and lay the groundwork for experimental research in this area.

Estimation of Winter Flounder Spawning Stock Abundance in the Niantic River

D. J. Danila

Northeast Utilities Environmental Laboratory
Millstone Nuclear Power Station
PO Box 128, Waterford, CT 06385

Abundance surveys of winter flounder spawning in the Niantic River, Connecticut have been made since 1976. These data have been used in the assessment of the operational impact of the Millstone Nuclear Power Station on this stock of winter flounder. Fish were obtained using otter trawls for mark and recapture experiments in which specimens larger than 15 or 20 cm were marked with a liquid nitrogen freeze brand for recapture in subsequent years. Absolute abundance estimates were made using the Jolly model for open populations. The trawl catch data were also used to calculate annual estimates of catch-per-unit-effort (CPUE). The Jolly absolute abundance estimates are considered reliable only since 1984 because of changes in sampling techniques and data collection methods, and both the Jolly estimate and CPUE are biased to some degree.

Population size ranged from about 36 to 80 thousand fish during 1984-90. A relative abundance index for female spawners, termed the annual standardized catch, was calculated from the trawl catches using abundance, gender, length-frequency, and maturity-at-size information. These annual indices made up a relatively consistent fraction (geometric mean of 3.5%; range of 2.7-4.5%) of the Jolly absolute population estimates. The relationship enabled the calculation of absolute female spawning stock size back to 1977 by extrapolation from the annual standardized catches, numbers of female spawners have ranged from about 19 to 79 thousand each year. The spawning population peaked in the early 1980s, as several large year-classes produced in the later 1970s were recruited to the spawning stock.

Growth and Maturation of Winter Flounder in Massachusetts Waters

D. Witherell[1] and J. Burnett[2]

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Growth and maturation of winter flounder were analyzed from data collected during the 1983-91 Massachusetts Division of Marine Fisheries spring inshore bottom trawl surveys. Data were analyzed separately for both Massachusetts stock units (north and south of Cape Cod). A von Bertalanffy growth model was fitted to length at age data for males and females of each stock unit. Analysis showed that winter flounder from the southern stock grew faster than those from the northern stock, and females grew faster than males. Mean lengths at age and calculated growth patterns for each sex were similar to those estimated from 1964-68 coastal tag return information. Annual variability in mean lengths at age was minimal, and was primarily due to changes in exploitation patterns resulting from regulations rather than changes in abundance or recruitment. Observed maturity at length data were well fitted by logistic models. Lengths at 50% maturity calculated from the curves were 28.5 and 29.3 cm for females, and 29.4 and 27.9 cm for males from the southern and northern stocks, respectively. Ages at 50% maturity were 3.1 years and 3.4 years for females from the southern and northern stocks, respectively, and 3.3 years for males of both stocks. These results show that Massachusetts winter flounder grow at faster rates and mature at larger sizes than has been reported for all other coastal populations.

The Reproductive Cycle in Winter Flounder off Newfoundland

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Newfoundland winter flounder spawn shortly after the end of their winter fast. If gametogenesis then occurs for the next spawning season, it is initiated for females during the summer feeding season with vitellogeneisis established, for Avalon Peninsular fish, by the end of August in recent years. Males undergo rapid spermatogenesis towards the end of the feeding season and hold activable sperm during the winter fast. Wild and laboratory held winter flounder may omit a spawning cycle in response to a poor feeding season.

Exploratory Simulation of Some Environmental and Biotic Factors Affecting YOY Winter Flounder Growth and Survival*

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YOY winter flounder population dynamics were modeled by individual-based Monte Carlo simulation. The model begins with the spawning of individual females, and follows each female-cohort of eggs through the yolk-sac stage until initiation of first feeding. At first feeding, individual larvae are sampled, and the growth and survival of individual feeding larvae are followed day-by-day through metamorphosis until the end of the year. Growth is represented with a bioenergetics model, with prey consumption resulting from stochastic prey encounters and captures. Survival of individual larvae is dependent on weight ("starvation") and length ("predation"). Niantic River and Narragansett Bay information was used to specify the environmental variables of temperature and prey types and densities. Model predictions under "typical" conditions were corroborated against Niantic River densities and length distributions. Exploratory simulations were performed comparing the effects on end-of-the-year survivors of: (1) number, sizes, and timing of spawning females, (2) interannual differences in daily water temperature, and (3) intensity of mortality during the larval period. Preliminary results suggest that varying combinations of these factors simultaneously can have interactive or synergistic effects on winter flounder dynamics during their first year of life.

*Research sponsored by the Electric Power Research Institute under Contract No. RP 2932-2 (DOE No. ERD-87-672) with the U. S. Department of Energy, under Contract No. DE-AC05-84OR21400 with Martin Marietta Energy Systems Inc.

Winter Flounder Trophodynamics in the Stressed New York Bight Apex

F. Steimle

National Marine Fisheries Service
Northeast Fisheries Science Center
Sandy Hook Laboratory
Highlands, NJ 07732

There is limited information of effects of sewage sludge disposal in coastal waters on trophodynamics of fishery resources. Possible effects include diet or feeding alterations in response to substances in the sludge or bottom water quality conditions, *e.g.,* hypoxia, promoted by sludge deposition and microbial degradation. During a three-year sewage sludge disposal cessation study in the New York Bight apex, stomach contents of 4,189 winter flounder were examined from bimonthly collections at three stations in the apex.

Results indicate a mixed response to cessation, with about a 100% increase in percent empty stomachs (a possible indicator of feeding inhibition) at the site closest to the former disposal areas and a 30 to 40% decrease at two stations several miles away. The composition of the diet showed no major changes in dominant prey (cerianthiid anemones, nemertians, and several larger polychaetes) during the study, although the percent frequency of occurrence (%FO) of *Capitella* sp. (a stressed environment indicator) decreased from means of 3-19% during disposal to <1% at all stations after cessation. The %FO of some amphipod species (possible indicators of relatively unstressed environments) increased from means of 0.7-6.7% during disposal to 4.9-36.8% after cessation.

These responses closely correspond generally to changes in the population abundances of these prey in the benthic community monitored during the study. All benthic community changes were not, however, reflected in the diet composition, *e.g.,* mollusc population variability. Preliminary data on chemical contaminant body burdens of major prey from the former disposal area and nearby areas suggest this factor is important and probably contributes to the chemical contamination of winter flounder tissue reported for the area.

Effects of Environmental Salinity and Thyroid Axis Manipulation on Survival and Growth in Juvenile Winter Flounder

T. A. Whitesel*, K. W. Able, and M. L. Keefe

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211 Inlow Hall, Eastern Oregon State College
LeGrande, OR 97850

This study examined the influence of environmental salinity and the thyroid axis on the survival and growth of juvenile winter flounder (*Pleuronectes americanus*). Metamorphic larval flounder were obtained on 28 March 1991, from the National Marine Fisheries Service (Milford, Connecticut) and reared at Rutgers Marine Field Station (Tuckerton, New Jersey).

The range of salinities which flounder could tolerate was examined in an initial set of experiments in May. When fish were directly transferred from ambient seawater (SW) (29-31 ppt) to 0, 1, 3, 4, 6, 7, 14, 21, 28 and 35 ppt SW, only fish in the 0 and 1-ppt treatment died within120 h, whereas fish in 6-35 ppt SW all survived for 120 h. Fish that were gradually acclimated to lower salinities began to die in 3.5 ppt SW and no fish survived at a salinity less than 1.75 ppt.

A second set of experiments examined the effect of manipulating the thyroid axis on the survival and growth of juvenile flounder over the range of salinity they could tolerate. Fish were transferred directly from 29 ppt SW to 2, 7, 21, and 35 ppt SW, as well as to a saline environment that fluctuated between 15 and 30 ppt (15/30) every 48 h; treated with 0.1 ppm thyroxine (T_4), 30 ppm thiouracil (TU) or as a sham (C); and held for either 120 h or 16 d. No effect of thyroid manipulation was seen on the survival in any salinity within 120 h. However after 16 d, treatment with TU increased the mortality observed in 7 and 35 ppt SW whereas mortality was minimal in the other treatments. After 16 d treatment with TU also enhanced the absolute growth of fish in 21 and 15/30 ppt SW whereas absolute growth was not affected by the other treatments.

These data confirm that juvenile winter flounder are not euryhaline but suggest they can survive and grow equally well in salinities ranging from 6 or 7 to 35 ppt. Furthermore, the effect of the thyroid axis on growth and survival appears to be modified in a complex manner by environmental salinity.

Questions on Variability in MFO Measurements from Wild Winter Flounder Stocks in Nova Scotia Waters

J. H. Vandermeulen, D. Mossman, and V. Vignier

Bedford Institute of Oceanography, Department of Fisheries and Oceans
Dartmouth, Nova Scotia, Canada B2Y 4A2

In the course of 5 years of MFO and related measurements (total cyt. P450) in winter flounder from several locations on the Scotian Shelf, we have encountered very wide swings in variability, including from year to year. Factors influencing this variability appear to be season, sex, state of maturity, and age. We have been able to maintain low enough levels of variability in our laboratory induction work to use both EROD and AHH (BaPH) as measures of environmental condition. Our observations raise questions about the use of MFO measurements in wild migratory fish stocks with unknown feeding and exposure histories.

Effects of Organochlorine Pesticides on Active Organic Anion Secretion by Primary Cultures of Winter Flounder Proximal Tubules*

M. A. Dawson[1, 2] and J. L. Renfro[2]

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Northeast Fisheries Science Center
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Marine/Freshwater Biomedical Sciences Center
Department of Physiology and Neurobiology
Storrs, CT 06268

A number of potentially toxic compounds, both endogenous and xenobiotic, are secreted into the urine as organic anions by the renal proximal tubule. Such secretion reduces the total body burden of these toxic substances; accumulation of certain organic anions in kidney cells and urine in the course of this secretion may subject the kidney to damage. We tested the potential of this system to transport four closely related organochlorine pesticides, using inhibition of p-aminohippuric acid (PAH) secretion as a criterion. The pesticides tested were 2, 4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4-dichlorophenoxy) propionic acid (dichloroprop), 2-methyl, 4-chlorophenoxyacetic acid (MCPA), and 2-(2-methyl, 4-chlorophenoxy) propionic acid (mecoprop). PAH flux was measured at a PAH concentration of 10 μM in the presence of 100 μM of each pesticide. Three of the four inhibited PAH flux at the latter concentration and were further tested at pesticide concentrations ranging from 0.1 μM to 1.0 mM. MCPA has no significant effect at 100 μM and was not tested further.

The use of cultures allowed us to mount the tissues in Ussing chambers and to measure unidirectional fluxes in the absence of electrochemical gradients. We monitored tissue concentration throughout each experiment by measurement of transepithelial potential difference (PD) and resistance (R). At the end of each experiment, as a further indication of tissue condition, we measured sodium-dependent glucose transport, as indicated by phloridzin-sensitive short circuit current (PSC).

2, 4-D at concentrations of 0.1 and 1.0 mM inhibited PAH secretion by 20% and 80%, respectively. Lower concentrations had no significant effect on secretion. No 2, 4-D concentration tested significantly affected reabsorption, PD, R, or PSC.

Dichlorprop inhibited PAH secretion at concentrations of 10 μM and above. Inhibition ranged from 19% at 10.0 μM to 77% at 1.0 μM. No dichloroprop concentration tested changed reabsorptive flux, PD, R, or PSC.

Mecoprop inhibited PAH secretion by 18% at 0.1 mM and by 80% at 1.0 mM with no effect on reabsorption, PD, R, or PSC. At mecoprop concentrations of 0.1 and 1.0 μM, PAH flux increased steadily through the experiment, reaching 139 and 132% of control, respectively, at 120 minutes. The simplest explanation, as yet untested, is that intracellular mecoprop accumulates and eventually increases PAH flux by anion exchange at the cytosolic face of either the peritubular or the luminal membrane. This biomonitoring system shows that these pesticides interact with anion secretion in the proximal tubule and may, through competition or stimulation, affect the dwell time of other toxins in the body.

*Research supported by NIEHS #ESO3848 and Connecticut Department of Environmental Protection #CWF228-R.

Antibody Levels Against Bacterial Pathogens are Increased in Winter Flounder from Polluted Sites

R. A. Robohm and D. Kapareiko

National Marine Fisheries Service
Northeast Fisheries Science Center
Milford Laboratory, Milford, CT 06460

The sera of 832 winter flounder, *Pleuronectes americanus*, from Long Island Sound and Boston Harbor were tested for antibodies against a panel of eight bacterial pathogens. Antibody titers were determined by agglutination reactions against formalin-fixed bacteria. The presence of antibodies (rather than non-specific agglutinins) was confirmed by treating representative sera with 2-mercaptoethanol, an antibody-disrupting reagent. To avoid a temperature effect that could bias comparisons of antibody levels between the 10 sites of fish capture, serum titers were divided into 2 groups: [1] those from fish captured at temperatures above 5ºC and [2] those from fish captured at 5ºC or less. Site comparisons for both temperatures indicated that total antibody levels are generally proportional to the estimated degree of pollution at the site. For example, the progression of calculated antibody indices for sites of fish capture when the temperature was 5ºC or less was: Shoreham < Niantic < Milford < Madison < Boston [DI] < Hempstead < Norwalk=Boston [LI]: the antibody indices for Boston [LI], Norwalk, and Hempstead were all significantly greater than the index for Shoreham. For temperatures above 5ºC the progression was: Niantic < Shoreham < Bridgeport < New Haven; the antibody indices for Boston [LI], Norwalk, and Hempstead were all significantly greater than the index for Shoreham. For temperatures above 5ºC the progression was: Niantic < Shoreham < Bridgeport < New Haven; the antibody indices for Niantic and Shoreham were significantly less than those for Bridgeport and New Haven. This work indicates that, in winter flounder, the presence of higher mean antibody levels (against selected bacteria) signifies residence in anthropogenically-degraded environments.

The Cytopathology of Fin Erosion Disease in Winter Flounder

J. E. Bodammer

URI/NOAA Cooperative Marine Education and Research Program (CMER)
University of Rhode Island, Kingston, RI 02881

High resolution light microscopic and fine structural studies were conducted on the early stages of fin erosion disease in winter flounder collected from Raritan Bay, the New York Bight, and New Haven Harbor, three severely contaminated sites along the Atlantic coast.

Fin tissues routinely demonstrated the following three types of lesions 1] frank necrosis resulting in severe epithelial sloughing; 2] epidermal hyperplasia (nonpapillomatous) with and without acantholysis; and 3] nonhyperplastic epithelia featuring middle-cell layer acantholysis accompanied by excessive accumulation of intracellular glycogen.

The underlying dermis also exhibited necrosis, blood vessel occlusion, and hemorrhage. A consistent absence of either bacteria or viruses in the epidermis and underlying lamina propria supports an idiopathic diagnosis for this disease at present; however, the putative effects of contaminants will be discussed.

Hydropic Vacuolation in the Liver of Winter Flounder

M. J. Moore and J. J. Stegeman

Woods Hole Oceanographic Institution
Biology Department
Woods Hole, MA 02543

Our previous studies of the development of liver disease in winter flounder, *Pleuronectes americanus*, from Boston Harbor revealed hydropically vacuolated cells to first appear in the biliary productular epithelia, which are found in the center of hepatic tubules. In larger and older fish, vacuolated cells were found throughout hepatic tubules and in grossly visible nodules. Vacuolated cells were intimately associated with a diverse array of cholangiocellular neoplasms, and the less common hepatocellular tumors. In this study, the ultrastructure and DNA synthetic activity of vacuolated cells was examined to investigate their potential role in the progression to neoplasia.

Ultrastructural studies showed that the development of vacuolation involved the accumulation of electronlucent material within the cisternae of the endoplasmic recticulum, in the perinuclear space, and in mitochondria.

The potential for these cells to proliferate was examined by developing an immunohistochemical assay to detect nuclei actively synthesizing DNA in S-phase. Flounder were pulse labeled with a nucleotide analog, bromodeoxyuridine (BrdU). Nuclear incorporation of BrdU was detected immunohistochemically. Vacuolated, and most particularly neoplastic nuclei, were found to have elevated numbers of nuclei that incorporated BrdU. It was concluded that this was evidence for replicative DNA synthesis, increased cell cycling, and possibly cell proliferation. The utility of the BrdU incorporation assay is currently being developed as an assay that will examine the linkage between epigenetic carcinogens and detrimental biological effects.

Risk of Disease in Winter Flounder of Northeast Estuaries

S. A. MacLean and C. Meise-Munns

National Marine Fisheries Service
Northeast Fisheries Science Center
Narragansett Laboratory
Narragansett, RI 02882

Data on histopathological conditions of winter flounder collected from 11 Northeast estuaries (from Great Bay, NJ to Casco Bay, ME) during the National Status and Trends program were analyzed for years 1984 through 1986. Lesions in the gill, kidney and liver of 809 winter flounder were categorized by type (e.g., inflammatory, degenerative, proliferative) according to NS&T standards. Using the prevalence of lesions, the odds ratio was calculated as a measure of potential risk of developing some types of lesions when factored against estuarine collection site (an estimate of contaminant exposure) and size (an estimate of age). Contaminant exposure increased risk of some lesions, as did age alone. Some lesions showed no risk associated with these two factors.

Abstracts
Poster Presentations

Cloning of Two P-glycoprotein Genes in Winter Flounder (*Pleuronectes americanus*)

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P-glycoprotein (P-gp) is a membrane-spanning protein, which is responsible for the energy-dependent efflux of drugs, called multiple-drug resistance (Mdr), observed in mammalian cell lines, tumors or cancer cells. Recent studies have shown that both P-gp and cytochrome P-450 genes were inducible in rat liver following administration of xenobiotics. We have isolated P-gp gene specific probes from the winter flounder to test the hypothesis that P-gp can be induced by xenobiotics as part of the detoxification process in fish.

Southern blot analysis using a conserved 3'-terminal region of hamster P-gp CDNA (pEXI) as a probe revealed that there are two P-gp genes in right-eyed flounders. We have isolated two sets of clones from a winter flounder genomic library that correspond to the 3'-ends of the two flounder P-gp genes. Sequence analysis has been focused on two key areas: the 3'-ATP binding site and the pEXI region, both of which are homologous with their mammalian counterparts. These cloned sequences are the first set of P-gp genes reported in fish and will be useful for delineating the expression of P-gp genes in teleosts.

Development of a Population Model for Anthropogenic Effects upon Reproduction, Growth, and Survival of Winter Flounder in Long Island Sound

Staff

National Marine Fisheries Service
Northeast Fisheries Science Center

Environmental quality has been theorized to affect the stability of winter flounder populations in highly urbanized coastal areas. We propose to test this hypothesis in Long Island Sound using flounder from environmentally contaminated New Haven Harbor and from a "clean" area off Milford, Connecticut. Recent research has demonstrated abnormal biological function in the flounder, including reproductive impairment, at New Haven. We will strengthen existing evidence by expanding current field and laboratory research, and by simultaneously developing a conceptual winter flounder life history model to evaluate the hypothesis. The use of a life history model will help to organize the available information on anthropogenic effects upon winter flounder. The model will aid in interpreting data, and will generate logical predictions on anthropogenic effects at the population level- a necessary prediction that has been elusive.

The proposed study will be a collaborative effort between two laboratories in the NMFS Northeast Fisheries Science Center. The Milford Laboratory will conduct field and laboratory studies designed to provide data on reproduction, growth and survival of winter flounder, especially as these parameters relate to toxic contamination and environmental degradation. The Woods Hole Laboratory will conduct age and growth analyses and will develop the life history/population model.

Numerical Response of Winter Flounder to Herring Spawn in the Southern Gulf of St. Lawrence

R. Morin[1], R. Tallman[2] and D. K. Cairns[1]

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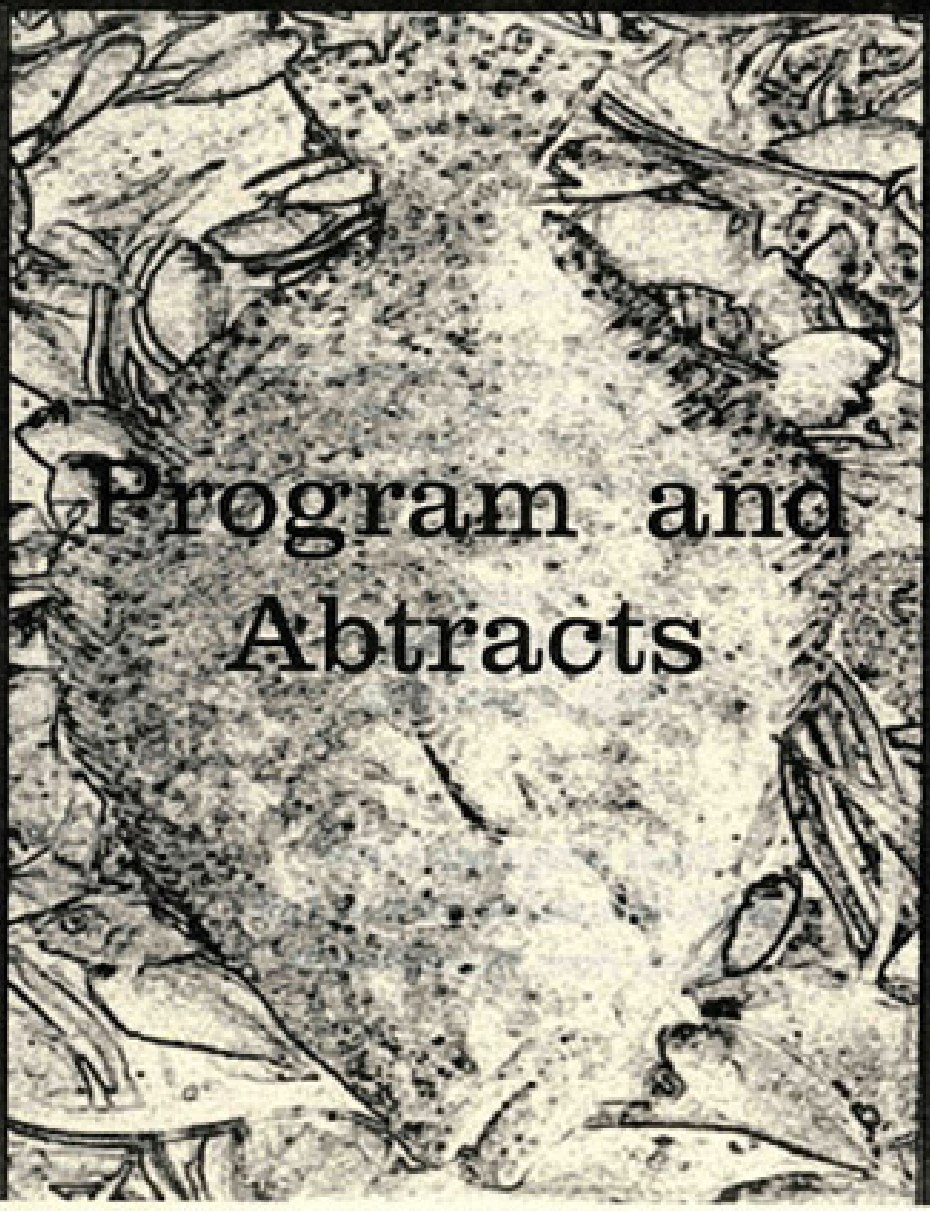
To test the hypothesis that a strong predator-prey linkage exists between winter flounder and herring spawn, we attempted to fit the Holling numerical response model to flounder predation on herring spawn deposited at Fisherman's Bank. P.E.I. Surveys using underwater video cameras and scuba divers demonstrated that we could predict both where and when herring would spawn. Interannual variation was limited to position of the spawning beds on the bank and fluctuations of a week or two in the start and end of spawning. Camera surveys showed that flounder were closely associated with deposited spawn. Examination of stomach contents revealed that flounder consumed large quantities of eggs. However, the density of flounder did not increase as a smooth function of the increasing density of herring eggs. Area trawl surveys revealed substantial numbers of potential flounder predators. We suggest that *in situ* study results do not fit a standard aggregative response pattern because flounder must trade off between increased vulnerability to predators when positioned on a patch of eggs and the pay-off from eating eggs. We propose that these results show that winter flounder are a major predator of herring spawn, but are constrained from achieving a maximal rate of harvest by the presence of predators. We also propose that herring may be concentrating reproduction in time and space to swamp potential predators. The successful evolution of mass spawning must have been accelerated because large spawning beds are risky places to operate as an egg predator.

National Marine Fisheries Service
Northeast Utilities

Flatfish Biology Workshop

December 6 & 7, 1994
Mystic, CT



Program and Abtracts

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Flatfish Biology Workshop
December 6-7, 1994, Mystic, Connecticut

by Conference Steering Committee: Anthony Calabrese (Chair)[1], Allan Beck[2], Jay Burnett[3], Donald Danila[4], Arnold Howe[5], Penelope Howell[6], Ambrose Jearld[3], and Anne Studholme[7]

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Fourth in a series of Flatfish Biology Conferences

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Flatfish Biology Workshop

December 6-7, 1994, Best Western Sovereign Hotel, Mystic, Connecticut

Oral Presentations

Tuesday, December 6th

7:45 a.m. **Registration/Coffee-Best Western**

8:45 a.m. Welcome and Introduction
Allen E. Peterson, Jr., Director
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, MA

Anthony Calabrese, Conference Chair
National Marine Fisheries Service
Northeast Fisheries Science Center
Milford, CT

Session I

Allan Beck, Chair
Environmental Advantage Group
Prudence Island, RI

9:00 a.m. The Development and Use of an *In Vitro* Assay to Evaluate Antigen/Antibody Responses in Winter Flounder
J. Bodammer
National Marine Fisheries Service/University of Rhode Island
Kingston, RI

9:20 a.m. An Idiopathic Lesion with Large Vacuoles in Hepatocytes of Winter Flounder from Boston Harbor and Massachusetts Bay
R. E. Hillman[1] and M. J. Moore[2]
[1]Battelle Ocean Sciences, Duxbury, MA and [2]Woods Hole Oceanographic Institution, Woods Hole, MA

9:40 a.m. Neutronal Adaptation Accompanying Metamorphosis in the Flatfish Suggests that an Evolutionary Plan Underlies Vertebrate Oculomotor Behavior
R. Baker[1] and W. Graf[2]
[1]NYU Medical Center, New York, NY and [2]Labortoire de Physiologie de la Perception et de l'Action CNRS, Paris, France

10:00 a.m. A Two-year Field Study of Vitellogenesis in Winter Flounder (*Pleuronectes americanus*) Under PAH-contaminated Conditions
J. M. Nicolas[1], J. H. Vandermeulen[2], D. Mossman[2], and S. Kirby[1]
[1]Dalhousie University, Halifax, Nova Scotia, Canada and [2]Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

10:20 a.m. **Coffee Break**

Session II

Don Danila, Chair
Northeast Utilities Environmental Laboratory
Waterford, CT

10:50 a.m. Predator-prey Interactions between Benthic Crustaceans and Recently Settled Winter Flounder: Field Observations
D. A. Witting and K. W. Able
Rutgers University, Tuckerton, NJ

11:10 a.m. Settlement Areas for Winter Flounder: Patterns in the Use of Coves Near Inlets
M. C. Curran and K. W. Able
Rutgers University, Tuckerton, NJ

11:30 a.m. Analysis of Life History Patterns in Flatfish with Emphasis on North Atlantic Species
C. Chambers
Huntsman Marine Science Centre, St. Andrews, New Brunswick, Canada

11:50 a.m. Distribution and Abundance of the Blackcheek Tonguefish, *Symphurus plaguisa* (Cynoglossidae: Pleuronectiformes), in Chesapeake Bay
M. R. Terwilliger
Virginia Institute of Marine Science, Gloucester Point, VA

12:10 p.m. **Hosted Lunch**

Session III

Penny Howell, Chair
Connecticut Department of Environmental Protection
Old Lyme, CT

1:10 p.m. Comparisons of Growth and Mortality in Juvenile Winter Flounder from New Haven Harbor and the Connecticut River, 1993
C. Meise[1], J. Widman[2], P. Howell[3], P. Clark[2], R. Goldberg[2], J. Hughes[2], R. Mercaldo-Allen[2], J. Pereira[2], D. Perry[2], and D. Nelson[2]
[1]National Marine Fisheries Service, Narragansett, RI, [2]National Marine Fisheries Service, Milford, CT, and [3]Connecticut Department of Environmental Protection, Old Lyme, CT

1:30 p.m. Distribution, Growth Rate and Estuarine Recruitment of North Carolina Summer Flounder Larvae
D. S. Peters, and H. J. Walsh
National Marine Fisheries Service, Beaufort, NC

1:50 p.m. Monthly Trends in Growth and Feeding in Captive Immature Winter Flounder (*Pleuronectes americanus*)
D. Mossman and J. H. Vandermeulen
Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

2:10 p.m. Growth Estimates for Larval and Juvenile Winter Flounder in Cape Cod Estuaries
K. L. Lang and F. P. Almeida
National Marine Fisheries Service, Woods Hole, MA

2:30 p.m. The Anisotropic Structure of Fish-scale Patterns and its Consideration in the Quantification of Seasonal Growth
I. Smolyar[1], F. Almeida[2], and J. Burnett[2]
[1]SES, Inc., Greenbelt, MD, and [2]National Marine Fisheries Service, Woods Hole, MA

2:50 p.m. **Cider Break**

Session IV

Ambrose Jearld, Chair
National Marine Fisheries Service
Woods Hole, MA

3:20 p.m. Preliminary Results of the Utilization of the Newport River Estuary, North Carolina, by Juvenile Flatfish Species
D. P. Cyrus[1], D. E. Hoss[2], and D. S. Peters[2]
[1]University of Zululand, Natal, South Africa and [2]National Marine Fisheries Service, Beaufort, NC

3:40 p.m. Prediction of Optimum Spawning Location and Interannual Variation in Year-class Strengths of the Hecate Strait Fishes in Relation to Hydrodynamic Effects on Larvae
F. Juanes[1] and C. J. Walters[2]
[1]University of Massachusetts, Amherst, MA and [2]University of British Columbia, Vancouver, British Columbia, Canada

4:00 p.m. Effect of Somatolactin and Related Hormones on Winter Flounder Renal Functions
M. Lu[1], J. L. Renfro[1], and P. Swanson[2]
[1]University of Connecticut, Storrs, CT, and [2]National Marine Fisheries Service, Seattle, WA

4:20 p.m. Spatial and Temporal Patterns of Abundance of Larval and Juvenile Windowpane Flounder, *Scophthalamus aquosus*, in an Estuarine/Inner Continental Shelf System
M. J. Newman and K. W. Able
Rutgers University, Tuckerton, NJ

4:40 p.m. **Poster Set-Up**

5:30 p.m. **Hosted Mixer and Poster Session**

Wednesday, December 7th

7:45 a.m. **Registration/Coffee**

Session V

Anne Studholme, Chair
National Marine Fisheries Service
Highlands, NJ

8:30 a.m. Fish Recruitment in the Northeastern United States: The Role of Estuarine Habitats
A. L. Studholme[1], A. Calabrese[2], K. W. Able[3], and S. C. Wainright[4]
[1]National Marine Fisheries Service, Highlands, NJ, [2]National Marine Fisheries Service, Milford, CT, [3]Rutgers University, Tuckerton, NJ, and [4]Rutgers University, New Brunswick, NJ

Habitat-specific Patterns for Distribution and Growth of Young-of-the-Year Winter Flounder in a Relatively Unimpacted Southern New Jersey Estuary
S. Hagan, R. Hoden, and K. W. Able
Rutgers University, Tuckerton, NJ

Habitat Use Patterns and Growth of Young-of-the-Year Winter Flounder in a Northern New Jersey Estuary
B. A. Phelan, J. T. Finn, A. J. Bejda, S. A. Fromm, D. Packer, and D. Jeffress
National Marine Fisheries Service, Highlands, NJ

Habitat Use Patterns and Growth of Young-of-the-Year Winter Flounder in a Central Long Island Sound Estuary in Connecticut
R. Goldberg, J. Pereira, P. Clark, F. P. Thurberg, and A. Calabrese
National Marine Fisheries Service, Milford, CT

Habitat Use and Growth of Young-of-the-Year Winter Flounder, *Pleuronectes americanus,* in the Lower Hudson River
J. M. Manderson[1, 2], A. L. Studholme[2], K. W. Able[1], and D. Packer[1]
[1]Rutgers University, New Brunswick, NJ, and [2]National Marine Fisheries Service, Highlands, NJ

Growth of Young-of-the-Year Winter Flounder in Three Estuarine Habitats as Measured by RNA/Protein Ratios
C. A. Kuropat, R. Mercaldo-Allen, and F. P. Thurberg
National Marine Fisheries Service, Milford, CT

Habitat Utilization by Juvenile Winter Flounder as Indicated by Stable Isotope Ratios
S. Y. Litvin and S. G. Wainright
Rutgers University, New Brunswick, NJ

10:00 a.m. **Coffee**

Session VI

Arnold Howe, Chair
Massachusetts Division of Marine Fisheries
Sandwich, MA

10:30 a.m. Influence of Hypoxia on the Foraging Ecology and Movement of Juvenile Winter Flounder, Windowpane and Fourspot Flounder
D. S. Lee and R. B. Whitlach
University of Connecticut, Groton, CT

10:50 a.m. Arsenic Induction of Metallothionein in Juvenile Winter Flounder
K. L. Jessen-Eller and J. Crivello
University of Connecticut, Storrs, CT

11:10 a.m. Utilization of New Haven Harbor as a Spawning and Nursery Area by Winter Flounder (*Pleuronectes americanus*)
J. J. Pereira, R. Goldberg, P. E. Clark, J. J. Ziskowski, R. A. Greig, J. B. Hughes, and D. M. Perry
National Marine Fisheries Service, Milford, CT

11:30 a.m. Habitat Models for Juvenile Pleuronectids Around Kodiak Island, Alaska
B. L. Norcross, F. -J. Muter, and B. A. Holladay
University of Alaska, Fairbanks, AK

11:50 a.m. A Multivariate Model of Winter Flounder Maturation: What's Age Got to do with it Anyway?
J. Burnett
National Marine Fisheries Service, Woods Hole, MA

12:10 p.m. **Hosted Lunch**

Session VII

Jay Burnett, Chair
National Marine Fisheries Service
Woods Hole, MA

1:10 p.m. Growth Rates at *ad libitum* Rations of Juvenile *Etropus crossotus* and *Citharichthys spilopterus* in North Carolina Coastal Waters
D. D. Davis[1], J. M. Miller[1], and J. S. Burke[2]
[1]North Carolina State University, Raleigh, NC, and [2]National Marine Fisheries Service, Beaufort, NC

1:30 p.m. Submersible Observations of Flatfish Nursery Habitats in the New York Bight
K. W. Able[1], R. K. Cowan[2], and M. P. Fahay[3]
[1]Rutgers University, Tuckerton, NJ, [2]State University of New York, Stony Brook, NY, and [3]National Marine Fisheries Service, Highlands, NJ

1:50 p.m. Biodiversity and Distributional Patterns of Symphurine Tonguefishes (*Symphurus*: Cynoglossidae: Pleuronectiformes) Occurring in the Western Atlantic Ocean
T. A. Monroe
National Marine Fisheries Service, National Systematics Laboratory, Washington, D.C.

2:10 p.m. Hudson/Raritan Estuary Winter Flounder
A. L. Pacheco, L. Stehlik, E. MacHaffie, and D. McMillan
National Marine Fisheries Service, Highlands, NJ

2:30 p.m. Salinity-related Changes in Resource Use by Two Sympatric Pleuronectid Flounders in Great Bay Estuary, New Hampshire
M. P. Armstrong
Massachusetts Division of Marine Fisheries, Sandwich, MA

2:50 p.m. Ontogeny of Functional Morphology of Feeding in Larval Fish: Winter Flounder *versus* Atlantic Cod
I. H. von Herbing and S. M. Gallager
Woods Hole Oceanographic Institution, Woods Hole, MA

3:10 p.m. **Adjourn**

Poster Session
Tuesday, December 6, 5:30 p.m.

Secretion of 2,4-Dichlorophenoxyacetic Acid (2,4-D) by Renal Proximal Tubule Primary Cultures is Inhibited by Phorbol Ester
P. A. Halpin and J. L. Renfro
University of Connecticut, Storrs, CT

Gonadotropin Induction of Testosterone and Estradiol-17β in Winter Flounder (*Pleuronectes americanus*) Ovarian Follicle Cultures
R. E. Gutjahr-Gobell[1], D. E. Black[2], and A. E. McElroy[3]
[1]SAIC, Narragansett, RI, [2]U. S. Environmental Protection Agency, Narragsentt, RI, and [3]State University of New York, Stony Brook, NY

Grossly Observed Lesions of Flatfishes from Various Northeast Embayments
S. A. MacLean[1] and J. J. Evans[2]
[1]National Marine Fisheries Service, Narragansett, RI, and [2]Maryland Department of Natural Resources, Oxford, MD

Heat Shock Stimulation of Daunomycin Secretion by Flounder Renal Proximal Tubule Cultures
C. Sussman-Turner and J. L. Renfro
University of Connecticut, Storrs, CT

Life History Aspects of Blackcheek Tonguefish, *Symphurus plaguisa* (Cynoglossidae: Pleuronectiformes), an Obscure, Abundant Flatfish in Chesapeake Bay and the Coastal Waters of Virginia
M. R. Terwilliger
Virginia Institute of Marine Science, Gloucester Point, VA

Validation of Daily Increment Formation in Juveniles of Four Flatfish Species: *Symphurus plagiusa*, *Scophthalmus aquosus*, *Paralichthys lethostigma*, and *Etropus crossotus*
M. J. Reichert, W. J. Jones, J. Seigle, and J. V. Campen
University of South Carolina, Columbia, SC

The Anisotropic Structure of Fish-scale Patterns and its Consideration in the Quantification of Seasonal Growth
I. Smolyar[1], F. Almeida[2], and J. Burnett[2]
[1]SES Inc., Greenbelt, MD, and [2]National Marine Fisheries Service, Woods Hole, MA

Analysis of Winter Flounder Population Structure Using Random Amplified Polymorphic DNA
T. L. Spinka, C. Callahan, K. Kallenbach, and K. Goddard
Ursinus College, Collegeville, PA

Distribution, Abundance and Habitat Preference of Juvenile Winter Flounder (*Pleuronectes americanus*) in the Shore-zone of Narragansett Bay, RI
C. Powell and R. Jones
Rhode Island Division of Fish, Wildlife and Estuarine Resources, West Kingston, RI

The Importance of Microzooplankton in the Diet of Winter Flounder Larvae
S. M. Gallager, L. Davis, and P. Alatalo
Woods Hole Oceanographic Institution, Woods Hole, MA

Marine Basins as Potential Winter Flounder Nursery Habitat
P. E. Pellegrino[1], M. E. Mroczka[2], and P. W. Dinwoodie[2]
[1]Southern Connecticut State University, New Haven, CT, and [2]Cedar Island Marina, Clinton, CT

Identification and Characterization of Cytochrome P-450s in Juvenile Winter Flounder
K. L. Wall and J. C. Crivello
University of Connecticut, Storrs, CT

A Novel Peptide Isolated from Flounder Skin has Antimicrobial Properties
G. Diamond and P. Weis
New Jersey Medical School, Newark, NJ

Maturation of Pleuronectiforms vs. Gadiforms: Size vs. Age
L. O'Brien
National Marine Fisheries Service, Woods Hole, CT

Abstracts
Oral Presentations

The Development and Use of an *In Vitro* Assay to Evaluate Antigen/Antibody Responses in Winter Flounder

J. E. Bodammer

URI/NOAA CMER Program
Fisheries, Aquaculture and Veterinary Science Department
University of Rhode Island
Kingston, RI 02881

During the past several years, we have been working on an *in vitro* assay method which would permit us to evaluate the antigen/antibody responses of winter flounder (*Pleuronectes americanus*) in both field and laboratory research. Using the well accepted passive hemolytic plaque assay (PHPA) as an analytical endpoint, we have aseptically isolated leukocytes from the flounder spleen, cultured the isolated cells in RPMI 1640 media, and antigenically stimulated them with the T-cell independent cell antigen TNP-LPS or the t-cell dependent antigen TNP-KLH before evaluating the number of plaque-forming (antibody producing) cells with TNP-labelled sheep red blood cells. This presentation will include technical information on the assay method and the results of applying it to the winter flounder immune system under laboratory conditions of stress (i.e., exogenous cortisol) or differing acclimation temperatures, as well as immunological data, obtained from fish collected in polluted (New Haven Harbor, New Haven, CT) and clean (Niantic River, Niantic, CT) sites in Long Island Sound.

An Idiopathic Lesion with Large Vacuoles in Hepatocytes of Winter Flounder from Boston Harbor and Massachusetts Bay*

R. E. Hillman[1] and M. J. Moore[2]

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Woods Hole, MA 02543

Winter flounder are collected annually in April from five sites in Boston Harbor and Massachusetts Bay as part of a monitoring study to assess the impact of the discharge of wastewater from the Deer Island Sewage Treatment Plant into Massachusetts Bay. The five sites include Deer Island flats, Broad Sound, off Nantasket Beach, the future outfall site, and a reference site in eastern Cape Cod Bay. Body burdens of selected organic and metal compounds in edible tissues and livers are determined, and histological observations of livers are made.

Livers of winter flounder collected in April 1993 contained a lesion previously unreported in fish from the northeast. The lesion was characterized by hepatocytes that had become swollen by a large vacuole that displaced the nucleus. The vacuole often contained a small round body with basophilic components. The presence of these cells in the liver was often accompanied by one or more pathological conditions including necrotic foci, loss of distinction of hepatocellular boundaries, cytomegaly, foci of basophilic or eosinophilic hepatocytes, and foci of cellular alteration. It is possible that the lesion is a manifestation of apoptosis, although other interpretations are possible at this time.

The lesion occurred in 59 percent of the fish collected on the Deer Island flats, the area of the present discharge from the sewage treatment plant; 62 percent of the fish from Broad Sound; 50 percent of the fish collected off Nantasket Beach; 52 percent of the fish collected at the future outfall site; and 23 percent of the fish from the reference site in eastern Cape Cod Bay. Although the Deer Island and Broad Sound sites receive the highest levels of anthropogenic input of contaminants, there was no correlation between any specific contaminant and the prevalence of the lesion.

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*This study is being conducted under a contract between Battelle and the Massachusetts Water Resources Authority.

Neutronal Adaptation Accompanying Metamorphosis in the Flatfish Suggests that an Evolutionary Conserved Plan Underlies Vertebrate Oculomotor Behavior

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Flatfish offer a natural paradigm to investigate adaptive changes in the CNS of vertebrates, since during metamorphosis the optic axis of each eye retains the same orientation in respect to the environment. Consequently, the vestibular reference frame is rotated 90º relative to the oculomotor coordinates. As a result, during swimming movements the same natural vestibular stimulation occurring before and after metamorphosis must produce different types of compensatory eye movements. This geometrical puzzle has been resolved by examining all of the anatomical and physiological changes that might occur extending from the sensory to motor periphery (i.e., from the labyrinths to the extraocular muscle apparatus). In summary, a unique synaptic connectivity was found in central vestibulo-oculomotor pathways of adult flatfishes wherein horizontal second-order vestibular neurons contacted vertical extraocular motoneurons. Extensive electrophysiological, immunohistochemical and ultrastructural observations revealed the new pathways to exhibit a reciprocal excitatory and inhibitory innervation of oculomotoneurons with characteristics common to that described in other vertebrates. From these data we conclude that the taxa-specific modifications of the adult flatfish vestibulo-oculomotor reflex are modeled after a representative vestibulo-oculomotor blueprint that appears to be highly conserved between vertebrates. Since the species studied to date are quite divergent on an evolutionary scale from a truly primitive vestibulo-ocular plan, we suggest that the development foundation for homologous canal-specific disynaptic excitatory-inhibitory pathways is the trait highly conserved through vertebrate phylogeny.

A Two-year Field Study of Vitellogenesis in Winter Flounder (*Pleuronectes americanus*) under PAH-contaminated Conditions

J. M. Nicolas[1], J. H. Vandermeulen[2], D. Mossman[1], and S. Kirby[1]

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The sediments of the Sydney Estuary are among the most contaminated along Canada's east coast. This is the result of almost 100 years of activity at a large coking facility located on the estuary's shoreline. Levels of up to 500 ppm of total sediment polycyclic aromatic hydrocarbon (PAH) have been reported (Vandermeulen, 1989). The chronic hydrocarbon contamination of Sydney Harbor has been an environmental concern for several years, but its effects on the reproductive biology of organisms exposed to the contaminants and the implications at the population level of organization had not been studied. Approximately 400 female winter flounder were collected in 1992 and 1993 over three sampling periods (June, September, November) from four stations in the estuary plus a control site. The stations were selected to reflect a gradient of sediment PAH concentrations ranging from 200 ppm (close to the point source) to less than detectable (control site). For each fish length, weight, age, egg and ovarian developmental stage, gonadosomatic index, hepatosomatic index, plasma estradiol and plasma vitellogenin were measured. Results show a significant effect of the sampling site on the reproductive parameters measured. The response of several of the parameters to contamination was non-linear, and suggests the existence of a threshold level of contaminant. The inconsistency of results over time and the implications for the flounder population of the Sydney Estuary are discussed.

Predator-prey Interactions Between Benthic Crustaceans and Recently Settled Winter Flounder: Field Observations

D. A. Witting and K. W. Able

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The relationship between benthic invertebrate predators and small benthic winter flounder (*Pleuronectes americanus*) in a well-defined settlement area (Holgate Cove, Little Egg Harbor, New Jersey) was determined based on stomach content analysis of shrimp and crab predators. Benthic invertebrates and juvenile winter flounder were sampled during a winter flounder settlement period (April-June 1992) with a 1-m beam trawl (6-mm mesh). Benthic flounder densities peaked in the cove in May with catches exceeding 10 m^{-2} at sizes of 7-32 mm SL, then decreased rapidly to <1 m^{-2} by the end of June when they ranged from 26-52 mm SL. The stomach contents of potential predators including *Calinectes sapidus* (8.3-123.7 mm, n=977), *Cancer irroratus* (10.0-47.3 mm, n=22), *Carcinus maenus* (18.3-70.7 mm, n=30), *Ovalipes occelatus* (9.1-36.8 mm, n=18), and *Crangon septemspinosa* (11.4-67.5 mm, n=2242) were examined for evidence of winter flounder predation (i.e., otoliths and vertebrae). Overall, 36 individual predators of three species: *C. sapidus* (n=30), *C. maenus* (n=2) and *C. septemspinosa* (n=4) had preyed upon winter flounder. *Calinectes sapidus* was the most common predator with an average of 4.3% (range 0-33%) of the individuals containing otoliths or vertebrae. Another crab *C. maenas* averaged 2.6% (0-33%), while *C. septemspinosa* had fewer otoliths (average 0.3%, range 0-5%). Back calculating the size of flounder prey based on an otolith-body length regression determined that most fish consumed by these benthic crabs and shrimps were between 8 and 41 mm SL. These data support the hypothesis that benthic crustacean predators, especially crabs, may impose significant levels of mortality upon settling and immediately post-settled winter flounder because although predation rates were low, the abundance of these predators was very high in the settlement area.

Settlement Areas For Winter Flounder: Patterns in the Use of Coves Near Inlets

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Coves near ocean inlets have been suggested to be important settlement areas for winter flounder (*Pleuronectes americanus*). The formation of a new cove in the Great Bay/Little Egg Harbor Estuary system in southern New Jersey during 1993-1994 gave us the opportunity to address this issue in more detail. Sampling in the same geographic location before it became a cove yielded no winter flounder. Upon cove formation, winter flounder density increased dramatically. To quantify winter flounder population size, a 1-m beam trawl with 3-mm mesh was used on 24 occasions during a 100-day period, with sampling intensity increasing during the period of maximum winter flounder density. The first individual (7.2 mm SL) was found in the cove on 7 May. Density peaked on 9 June (5 m^{-2}), with individuals ranging from 12.4-54.8 mm TL, and declined to a value of 0.2 m^{-2} on 19 August (size range 44.6-72.5 mm TL). Using regression analysis on the mean size of winter flounder per sampling date, the fish grew 0.6 mm day^{-1}. This result falls within the range of growth rates obtained in a complimentary study in which growth rates were calculated on caged flounder in other natural habitats in the same estuarine system. Approximately 1500 juvenile winter flounder from 13-72 mm TL were tagged in the cove to determine retention in and emigration from the cove. Fifteen fish were recaptured; the maximum duration of a marked fish in the cove was 20 days. This evidence, as well as the decrease in population size, and the small number of individuals over 45 mm TL, indicates that the cove is probably utilized for only 2-3 months immediately following settlement. Winter flounder strongly selected this site, even more so than a cove situated slightly farther from the inlet that had been an important settlement site in previous years. The new cove was the only site at which we found recently settled winter flounder in 1994. Further investigation into the temporal stability of coves and their function in winter flounder recruitment is warranted in light of the present study.

Analysis of Life History Patterns in Flatfish with Emphasis on North Atlantic Species

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We used data from literature sources to construct a quantitative description of flatfish life histories. Data were compiled from over 120 species of flatfish and were used to represent the range of across-species variability in a suite of important life history features, e.g., sizes and durations of pre-adult life stages, growth rate, maturation schedule, fecundity, and life span. The set of species resident in the North Atlantic were isolated and evaluated with respect to their coverage of "flatfish life history space". Where data were lacking for North Atlantic species, congeners or other species with the most similar life histories were identified. These similar species may be used as surrogates to provide first approximations of the life history and vital rates that we might expect for species where data are scarce and/or anticipated to be difficult to collect. The utility and limitations of the comparative approach are described.

Distribution and Abundance of the Blackcheek Tonguefish, *Symphurus plagiusa* (Cynoglossidae: Pleuronectiformes), in Chesapeake Bay

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Monthly trawl survey cruises were conducted in the lower Chesapeake Bay during 1955-1993 to produce indices of juvenile abundance of important marine and estuarine finfish and crustaceans. Data from 1986-1993 cruises can be used to determine distribution and abundance of blackcheek tonguefish in Chesapeake Bay and its tributaries: the York, James, and Rappahannock Rivers. Blackcheek tonguefish are the second most abundant pleuronectiform occurring throughout Chesapeake Bay and its tributaries, second only to hogchokers in abundance. It was found that blackcheek tonguefish are an extremely euryhaline species, as individuals were collected in waters ranging in salinity from 0.00 ppt to 36.2 ppt. Individuals were collected in depths ranging from 3.0 meters in the James River to 40.5 meters in the lower Chesapeake Bay. Abundance of blackcheek tonguefish was at a maximum in 1989 when 7156 specimens were collected. Abundance was at a minimum in 1993, with 1982 specimens reported.

Comparisons of Growth and Mortality in Juvenile Winter Flounder from New Haven Harbor and the Connecticut River, 1993

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Field estimates of growth and mortality for juvenile winter flounder from New Haven Harbor and the Connecticut River were made in 1993. Growth of juveniles was compared using the Gompertz growth equation. New Haven Harbor had higher growth constants than the Connecticut River. Mortality rates were also higher in New Haven Harbor (approximately 10%). How these increases in mortality affect fishable population numbers was explored.

Distribution, Growth Rate and Estuarine Recruitment of North Carolina Summer Flounder Larvae

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Larval density, spatial and temporal distribution, and otolith increment counts were determined from samples collected on transects across the continental shelf in Onslow Bay, and in areas immediately inside and outside of Beaufort Inlet. We found little evidence supporting a strong vertical stratification of larvae. The least developed larvae (about 3 mm SL with 3 to 5 otolith increments) were found on the middle shelf at 40 to 74 m depth. The larval size and increment count increased, to 6-10 mm SL with 15-20 increments, at the more shoreward stations. The highest densities (2-3/100 m^3) were found at intermediate depths of 20 to 30 meters. Fish immediately outside the inlet were more variable in size and increment number than those which were collected inside. Outside they ranged from 4.5-14 mm with 8-56 increments with a mode of about 12 mm and 34 increments. Inside the inlet, fish ranged from 10-18 mm with a mode of 14 mm, and increment counts ranged from 28-54 with a mode of 56. Based on the temporal occurrence of small larvae in shelf waters and the number of increments on larvae entering Beaufort Inlet, spawning season appears to extend from late November or early December into March. Length (mm) of all larvae captured = 2.8 + 0.26 number of increments, which is faster growth than observed in laboratory measurements.

Monthly Trends in Growth and Feeding in Captive Immature Winter Flounder (*Pleuronectes americanus*)

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Immature winter flounder (*P. americanus*) were obtained by otter trawl from a local coastal population. Each fish was tattooed on the blind side with an identification code. Three groups were held captive in 6-foot diameter circular tanks under ambient light and water temperature for a period of eleven months. Initial densities of 2.69, 1.20, and 0.766 kg/m^2 were established in each tank, respectively. Fish were fed a diet of chopped squid *ad libitum* every two days. Growth patterns in each of the tanks were monitored relative to water temperature, hours of daylight, and effects of density. Monthly length and weight measurements of individuals were recorded. Amount of food consumed (per week, per fish/day) was calculated for each tank. Fish condition index, simple growth rates and gross conversion efficiencies were determined. Food consumption and growth rates showed strong correlation with water temperature and daylight length. Final loadings in the three tanks were measured to be 4.24, 2.46, and 1.87 kg/m^2, accounting for total weight increases of 57, 105 and 144%, respectively. Effects of density and competition are investigated and statistical differences are discussed. Both positive (length and weight gain) and negative (weight and length loss) growth periods were observed in each tank. The latter occurred during the winter months (January to March). Caudal fin erosion and measurement error can only account for a small portion of the recorded "shrinkage" in length associated with periods of negative growth.

Growth Estimates for Larval and Juvenile Winter Flounder in Cape Cod Estuaries

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There have been numerous investigations into age and growth rates of winter flounder, *Pleuronectes americanus*, particularly in the juvenile stage. Our studies were conducted to provide estimates of growth of larval and juvenile winter flounder from a relatively "clean" environment for use in modeling the early life history and for comparative studies of habitat use by this species.

Separate field studies were conducted on the two life stages. In the first, approximately 2,000 larvae were exposed to oxytetracycline (OTC) at 500 mg/L for 24 hours and transferred to two floating 505-µ mesh cylindrical enclosures (1,000 larvae/enclosure). The enclosures, which measured 1 m in diameter and 2.5 m in length, were deployed in Waquoit Bay, MA for approximately one month. Larvae were sacrificed weekly to examine growth and validate daily growth increments.

For the study of juveniles, 12 fish were exposed to OTC (500 mg/L for 72 hours), and placed in four 1-m^2 subtidal enclosures (3 juveniles/enclosure) located adjacent to Ram Island within Great Harbor, Woods Hole, MA. These fish were marked with acrylic paint injections to identify individuals; one fish of each color was placed in an enclosure. Monitoring was done *via* videotape at ten-day intervals.

We found that larval winter flounder deposit increments on their sagittal otoliths daily (or nightly); a daily growth rate was calculated. We also found that otolith growth was decoupled from somatic growth in juvenile winter flounder and calculated both individual and population growth rates for these fish. The growth rates of both life stages will be combined for use in a life history model for this species.

The Anisotropic Structure of Fish-scale Patterns and its Consideration in the Quantification of Seasonal Growth

I. Smolyar[1], F. Almeida[2], and J. Burnett[2]

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Fish scale patterns are divided into seasonal growth zones with each zone providing information about variability of the fish's growth rate. The data derived from these scales are used in life history studies of fish. In our approach, the information about seasonal growth zones is derived from a plot describing relative widths of the spaces between circuli from the center of the scale to its margin. The shape of this plot is highly dependent on the direction of the measurement chosen due to numerous breaks and confluences of the circuli. Thus, scale patterns are anisotropic objects. Presently, there is no formal procedure for quantifying seasonal growth zones that takes into account the anisotropic nature of the scale pattern.

The objective of this study is to develop a formal procedure to quantify seasonal growth zones, which incorporates the anisotropic nature of scale patterns. The tool for solving this problem is a N-dimensional model of the scale pattern. This model is based on a table that describes the width of the circuli spacing in the N directions and a plot that represents the structure of the circuli such that all breaks and confluences are taken into account.

Plots of the relative widths of the spaces between circuli were plotted for N directions. The plot was used to combine patterns for different directions into a common chart. The resultant chart described the variability of the circuli for an entire scale. A Boolean function was used to define the notion of the "stability of circuli widths" in a context of the variability of the scale structure. The notion was introduced in order to investigate the impact of the anisotropic structure of scale patterns on the accuracy of the quantification of the seasonal growth zones and to help in the selection of the version of the circuli structure.

Scales of yellowtail flounder, *Pleuronectes ferrugineus*, of different ages were analyzed. The results showed that the method reduced uncertainty in the recognition of the seasonal growth zones as well as the quantification of the age of these fish. Further studies to quantify variability in growth patterns between fish collected from different areas, representing potentially different stocks, are underway.

Preliminary Results on the Utilization of Newport River Estuary, North Carolina, by Juvenile Flatfish Species

D. P. Cyrus[1], D. E. Hoss[2], and D. S. Peters[2]

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The role of the Newport River Estuary in the juvenile phase of the life cycle of flatfish species occurring along coastal North Carolina was determined using a microhabitat approach. The system was divided into six zones according to two dominant physical characteristics. These were salinity and turbidity, both of which exhibit strong gradients extending up the length of the system as well as showing seasonal variations. The microhabitats within each zone were identified and monthly sampling of each was undertaken. This took place from April, just after settlement, through to November, when the bulk of juveniles of marine species emigrate to the coastal waters. Although the target species of study were the Paralichthid flounders, whose adults are of importance in North Carolinas offshore fishery, data were collected on a total of nine flatfish species recorded. In addition, a series of physical measurements were taken each sampling.

Preliminary results show that the three Paralichthid species present are largely separated by microhabitat utilization, but that this was also influenced by other physical aspects, in particular salinity and turbidity. Their distribution changed during midsummer when salinities increased to >35 ppt, at which point the bulk of individuals left the system. At the same time, a dramatic increase in numbers of Etropus crossotus, particularly in the sand and compacted mud habitats, took place. Two other species, Symphurus plagiusa and Trinectes maculatus, were common and occurred throughout the system. These two are essentially estuarine species that are able to complete their entire life cycle within the system; however, distinct microhabitat utilization took place based on size.

Prediction of Optimum Spawning Location and Interannual Variation in Year-class Strengths of Hecate Strait Fishes in Relation to Hydrodynamic Effects on Larvae

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An individual-based model of larval drift and growth in relation to wind- and tide-driven advection and stratification in the Hecate Strait (British Columbia) was used to predict spatial and interannual variation in larval fitness. The model correctly predicted the main spawning location for English sole and Pacific cod, and indicated that this location represents a balance between access to better feeding conditions in the northern part of the strait versus export from this area due to wind-driven currents. However, the model was unable to predict observed patterns of interannual variation in year-class strength; it predicted more variable recruitment than has been observed. This failure could be due to incorrect estimates of spawn timing in relation to hydrodynamic conditions, omission of some key factor limiting larval survival, or strong control of survival by factors operating after larval settlement.

Effect of Somatolactin and Related Hormones on Winter Flounder Renal Functions*

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Prior studies by others have shown that somatolactin is produced in the pars intermedia of the pituitary gland by teleosts, including flounder, cod, and salmon. The target and function of the hormone have been uncertain. Winter flounder renal proximal tubule primary monolayer cultures mounted in Ussing chambers were used to determine the effect of salmon somatolactin (sSL) on transepithelial inorganic phosphate (Pi) and Ca^{2+} transport. sSL stimulated P^i reabsorption in a dose-dependent manner at physiological levels of the hormone (12.5 ng/ml). Net P^i transport was significantly altered by sSL (200 ng/ml) within 2 h after the initial exposure. Ca^{2+} fluxes were unchanged by the addition of 200 ng/ml sSL. The sSL-induced P^i reabsorption was abolished by 10 µM H-89, a highly specific protein kinase A inhibitor. Moreover, the production and release of a cAMP were significantly increased following 1 h and 2 h exposure to sSL. The data indicate that sSL directly stimulates net renal P^i reabsorption by a cAMP-dependent pathway. In addition to sSL, flounder SL and rat prolactin greatly, and salmon growth horomone (2.3 µg/ml) slightly, increased net P^i reabsorptive flux, whereas salmon prolactin had no effect. These data provide the first evidence for a physiological action of somatolactin, and together with evidence from other studies may implicate the hormone in renal nutrient conservation during gonadogenesis.

*Supported by the National Science Foundation

Spatial and Temporal Patterns of Abundance of Larval and Juvenile Windowpane Flounder, *Scophthalmus aquosus*, in an Estuarine/Inner Continental Shelf System

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The windowpane flounder, *Scophthalmus aquosus*, ranges from the Gulf of Saint Lawrence to Florida, but the species is most abundant from Georges Bank to Chesapeake Bay. Spawning is bi-model off of New Jersey and occurs from April to May, and again in September to October; however; the spatial and temporal distribution of planktonic larvae and newly settled and older juveniles has not been examined in detail.

Within the Great Bay/Little Egg Harbor estuarine system, annual variability in abundance existed between the spring-spawned cohorts such that in 1989 and 1993 windowpane abundances were much greater than they were in 1990, 1991, and 1992. These spring-spawned larvae ranged from 2.5-18.4 mm SL (n=280). Over these same years, the fall-spawned larvae were rarely collected inside the estuary and these larvae ranged from 7.4-13.5 mm SL (n=5). Differences in abundance between spring- and fall-spawned larvae from 1991 to 1992 were not apparent at an adjacent inner continental shelf site (LEO-15). These spring- and fall-spawned larvae ranged from 2.1-8.8 mm SL (n=90) and 2.3-9.2 mm SL (n=83), respectively, thus the maximum size of larvae at LEO-15 was much smaller than those collected in the estuary. This pattern may suggest that larger larvae from the spring cohort are moving into the estuary; however the fate of larger larvae from the fall-spawned cohort is unclear.

Newly settled spring-spawned individuals appear to use higher salinity, sandy portions of the estuary as nursery habitats throughout the summer where they reach a maximum size of approximately 63 mm SL by August. Evidence suggests that fall-spawned individuals are using similar estuarine habitats in the spring. Both cohorts also occur at LEO-15. These preliminary observations suggest that windowpane use the inner continental shelf and lower portions of estuaries as spawning and nursery areas in New Jersey.

Fish Recruitment in the Northeastern United States: the Role of Estuarine Habitats

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A comparative approach to assess the functional value of selected habitat types as nursery areas for juvenile fishes has been adopted in three southern New England-mid-Atlantic coast estuaries. Habitat use patterns are identified by comparing distribution, abundance and growth of newly-settled fishes in Great Bay-Little Egg Harbor and the Hudson-Raritan estuary in New Jersey, and Clinton Harbor along the Connecticut coast of Long Island. While each of these systems serves as a nursery area for a variety of juveniles, particularly winter flounder, *Pleuronectes americanus*, and tautog, *Tautoga onitis*, the availability of specific habitat types varies within and between systems, as does the degree of anthropogenic disturbance. The study has several objectives. The first, based on the premise that variation in growth may be an important indicator of habitat quality, is to determine habitat-specific growth rates, using several measures of growth, including somatic growth, change in otolith increments and RNA/protein ratios. A second objective is to determine spatial and temporal changes in habitat use patterns both qualitatively and quantitatively. A third objective is to examine trophic linkages of YOY fishes within specific habitats using stable isotope ratios of carbon and nitrogen. The first year of the study has been completed and results will be presented particularly as they relate to YOY winter flounder distribution, growth and trophic associations.

Habitat-specific Patterns for Distribution and Growth of Young-of-the-Year Winter Flounder in a Relatively Unimpacted Southern New Jersey Estuary

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In an attempt to define the relative quality of estuarine habitats for young-of-the-year winter flounder (*Pleuronectes americanus*), we determined the habitat-specific patterns of 1) distribution and abundance with traps and beam trawls and 2) growth with caging experiments. These observations and experiments were conducted in the Great Bay-Little Egg Harbor estuary, a relatively unimpacted, polyhaline, shallow (average depth < 2 m) system in southern New Jersey. During spring and early summer of 1994, early benthic-phase juveniles (< 30 mm TL) were found in settlement areas as described in our earlier presentation. After dispersing from these sites, winter flounder (20-103 mm TL) were subsequently collected in a variety of shallow vegetated and unvegetated habitats based on trapping and beam-trawl catches.

Survival and growth varied with habitat type. In salt marsh creeks, where dissolved oxygen levels were very low, survival was poor. In all other habitats, growth of caged individuals (14-73 mm TL) ranged from 11.1 to < 0 mm TL per day. The greatest growth rates occurred for the smallest individuals in early June and these rates declined significantly thereafter. Comparisons among habitats indicate that growth rates were similar except for marsh creeks, which were much lower. Together these data suggest that both vegetated and unvegetated habitats are important as nurseries for young-of-the-year winter flounder and these data agree generally with the results of an earlier study in the same estuary. Interpretation and extrapolation of these data should be made cautiously because we have 1) an inadequate understanding of movements and 2) movements between habitats.

Habitat Use Patterns for Growth of Young-of-the-Year Winter Flounder in a Northern New Jersey Estuary

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The Navesink River, located in the Hudson-Raritan estuary, has a variety of habitat types utilized by young-of-the-year fishes. Of these, three were selected for study: (1) unattached macroalgae (usually *Ulva lactuca*), which is a dominant habitat; (2) eelgrass (*Zostera marina*), which is scarce and patchy and (3) marsh creeks, which are usually anthropogenically altered. Habitat use patterns were determined both quantitatively (1-meter beam trawl) and qualitatively (fish traps). Habitat specific growth was measured in caging experiments. Data will be presented on habitat specific abundance, distribution and growth of young-of-the-year winter flounder (*Pleuronectes americanus*) in vegetated (macroalgae, eelgrass), adjacent unvegetated and in marsh creek habitats.

Habitat Use Patterns and Growth of Young-of-the-Year (YOY) Winter Flounder in a Central Long Island Sound Estuary in Connecticut

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The study area, in Clinton, Connecticut, has several habitat-types including: sea lettuce (*Ulva*) in Clinton Harbor, eelgrass (*Zostera*) in the Hammonassett River, and marsh creeks leading into Hammonasset State Park. Monthly trawling and mapping surveys and cage experiments were conducted to determine site-specific habitat use by and growth of winter flounder during their first year of life.

Preliminary trawling results indicate that young-of-the-year flounder (YOY) appeared in June with dispersal limited to primary settlement areas. By July, most YOY flounder were associated with eelgrass and adjacent unvegetated sites. In August, significant numbers of YOY were found in both eelgrass and sea lettuce sites. In August, several YOY flounder were sampled for the first time in one of the two marsh creeks. Trapping results were not always consistent with trawl samples at similar times or sites. Winter flounder were sampled in traps, but usually in low numbers.

Three ten-day cage experiments using YOY winter flounder were conducted between late June and early August. Recovery of flounder used in the first cage experiments (initial size about 35 mm total length) was poor and showed minimal growth, a result perhaps of stress from the initial capture, holding, and deployment into the experimental cages. Recovery of fish from the second flounder run (initial total length, about 45 mm) improved to about 50% and growth of up to 3.5 mm over 10 days was recorded for several individuals. Fastest growth was recorded among fish in cages in and near eelgrass beds. Initial total length of YOY flounder in the third run was about 55 mm. Recovery from this experiment exceeded 50%; however, growth was minimal, indicating either a seasonal drop in growth rate or inadequate nutritional conditions. In all three cage experiments, few fish were recovered from one of the marsh creek sites and none were recovered from a second. Data collected in August, using a continuous data logger, indicated diurnally low dissolved oxygen concentrations (< 2.0 ppm) and high water temperatures (> 27ºC) associated with low tides, conditions most likely detrimental to young fish.

Habitat Use and Growth of Young-of-the-Year Winter Flounder, *Pleuronectes americanus*, in the Lower Hudson River

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Trapping and growth experiments were performed in the lower Hudson River to evaluate the effects of artificial structures, particularly pile-supported platforms (piers), on the growth and distribution of recently-settled fishes. Study sites were established at subtidal depths (≤5m) in pile field, interpier, and underpier habitats on the eastern and western sides of the river in Manhattan, NY and Hoboken, NJ.

In June-October 1993 and May-September 1994, bimonthly trapping experiments were performed to determine the relative abundance of juvenile fishes in the three habitat types. Based on collections of 1474 individuals representing 25 species, the relative abundance and species richness of juvenile fishes was low in underpier habitats when compared with interpier and pile field habitats. Young-of-the-year winter flounder (12-141 mm TL) accounted for 2.2% of the total catch (N=15) in 1993, and for 10.5% (N=80) in 1994. In 1994, the abundance of winter flounder was relatively high in both interpier and pile field habitats (x cpue 0.116-0.166 fish d^{-1}) while fish collected in 1993 were caught exclusively in pile field habitats (x cpue 0.04-0.05 fish d^{-1}). Only one winter flounder was caught in an underpier habitat during the study.

Caging experiments (N=6) were performed on recently-settled winter flounder (N=522; 17.1-89.9 mm TL) in July-August 1993 and June-July 1994 to determine their relative growth rates in the three habitat types. During the study, 70% (N=365) of the fish caged were recovered live after the 10-day experiments. The mean instantaneous growth in weight of winter flounder caged in underpier habitats was negative in all six experiments. Instantaneous growth rates in length of fish caged in underpier habitats were negative or slightly positive. Mean growth rates in length or weight were positive for winter flounder caged in pile field habitats in three experiments and for fish caged in interpier habitats in four experiments. Mean growth rates as high as 5.0% and 1.5% d^{-1} in weight and length, respectively, were observed in the pile field and interpier habitats. The results of the trapping and growth experiments suggest that pile-supported platforms provide relatively poor habitat for recently-settled winter flounder when compared with interpier and pile field habitats.

Growth of Young-of-the-Year Winter Flounder in Three Estuarine Habitats as Measured by RNA/Protein Ratios

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A comparative study to assess the functional value of selected types of nursery areas for juvenile fish has been initiated in two estuaries in New Jersey and one in Connecticut. One method of measuring comparative fish growth between these estuaries is determination of RNA/protein ratios in fish muscle tissue.

Deoxyribonucleic acid (DNA) is found in constant quantities in normal somatic tissue within a given species, while ribonucleic acid (RNA) is present in variable quantities in the nucleus and cytoplasm. Quantities of RNA vary directly with the activity of protein synthesis: it is expected to be more concentrated in tissues undergoing faster growth or protein synthesis. Protein content of muscle tissue is an index of nutritional condition. Protein plays a major role in energy storage, metabolism, and is an important structural component of tissue.

Trial runs of the RNA/DNA procedure at Milford with juvenile winter flounder showed protein contamination of the DNA fraction. This is known to occur in juvenile fish and is not a result of the methodology. The relationship between cell number and size breaks down in juveniles, which contain fats and carbohydrates that contribute to body weight without adding cells. We therefore limited our analysis to the RNA fraction and expressed this information as a concentration of mg wet weight and/or mg protein. This is a more accurate and appropriate means of expressing growth in juvenile fish than the RNA/DNA ratio.

The results obtained from young-of-the-year winter flounder held in cages at sites in coastal areas of Connecticut and New Jersey will be presented.

Habitat Utilization by Juvenile Winter Flounder as Indicated by Stable Isotope Ratios

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The measurement of stable isotopes in tissues of marine consumers can allow a time-integrated estimation of assimilated diet. The nitrogen isotopic composition of a consumer is used as an indication of trophic level while values of ä^{12}C are employed to identify sources of organic carbon to consumers. Taken together, ä^{13}C and ä^{15}N data provide a two-dimensional view of the trophic position of juvenile winter flounder within an environment. In order to quantify the relationship between diet and consumer isotopic composition juvenile winter flounder were confined to cages for ten days in four different environments (marsh creek, sand bottom, benthic macroalgae, and *Zostera*). The juvenile fish and environmental samples consisting of sediment, *Ulva*, and *Zostera* were analyzed for C and N stable isotopes. These results yield information on the effects of confinement to a single habitat (and diet) on stable isotope composition and growth rate (RNA/DNA). In the future comparison of stable isotopes and RNA/DNA data from wild-caught fish with those raised under confined conditions (the present study) will allow us to determine habitat utilization by juvenile winter flounder in the field.

Influence of Hypoxia on the Foraging Ecology and Movement of Juvenile Winter, Windowpane, and Fourspot Flounder*

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A two-year study to examine the relationships between benthic community structure and secondary production, seasonal hypoxia, and the foraging ecology of demersal fishes was conducted in 1992 and 1993 near Branford, CT and Hempstead, NY. The Hempstead site was consistently exposed to hypoxic events while the Branford site remained normoxic throughout the summer. Periodic sampling of the benthic community, hypolimnetic oxygen, and fish stomach contents every 14 to 17 days was conducted in concert with the CT DEP's summer survey trawls. The diet composition of winter flounder reflected both benthic community structure and the effect of hypoxia on the distribution of benthic infauna at the Hempstead site. At both sites, diet composition changed predictably with fish size. During hypoxia, winter flounder larger than 100 mm TL were absent from the Hempstead site but remained abundant at the Branford site. However, juvenile winter flounder (50 to 99 mm) were still encountered at the Hempstead site during hypoxia. Foraging opportunistically on *Nepthys* and other polychaetes forced to the surface of the sediment by high sulfide and low oxygen concentrations, juvenile winter flounder moved in and out of the site in response to differential oxygen concentrations above and below the thermocline. Juvenile windowpane flounder foraged predominantly on copepods, *Crangon septemsinosus* and *Neomysis americanus*, crabs (*Pinnixia, Rithropanopeus, and Cancer*) and fish. All size classes of windowpane and fourspot flounder were absent from the experimental site during hypoxic events.

*This work was supported by the Connecticut Department of Environmental Protection's Long Island Sound Research Fund.

Arsenic Induction of Metallothionein in Juvenile Winter Flounder

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Tissue background levels of arsenic were unusually high in winter flounder (Pleuronectes americanus) collected in Long Island Sound. Juvenile flounder (length 100-150 mm) from the relatively clean Niantic River/Bay region had liver arsenic concentrations between 2.0-273.0 µg/g liver. Although arsenic induction of the metal-binding protein metallothionein (MT) has been demonstrated in mammals, this is the first study to show such a relationship in fish. An arsenic liver uptake curve was generated by exposing juvenile winter flounder to sodium arsenite (2.0, 4.0, 8.0 and 20.0 µM/g fish) via subcutaneous implants. The liver arsenic levels of all treatments differed significantly ($p < 0.01$) from the control after 24 hours. The treatments also exhibited a pattern of arsenic accumulation with increasing dosage. No fish died at the lowest dose, however 10%, 20%, and 40% mortality was observed at the respective higher levels. Subsequent determination of MT in surviving fish showed that it was significantly ($p < 0.01$) elevated above the control for each arsenic treatment. However, no increasing pattern of MT protein was evident beyond the first treatment. These results suggest that arsenic does induce fish MT but more research is necessary to determine an MT/arsenic dose-response curve. Future work will include evaluation of MT protein at lower levels of exposure. The time course of MT protein and message induction in fish by sodium arsenite will also be examined.

Utilization of New Haven Harbor as a Spawning and Nursery Area by Winter Flounder (*Pleuronectes americanus*)*

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The presence of winter flounder larvae, juveniles, and gravid adults in New Haven Harbor and the Quinnipiac River has been noted by various investigators without linking them into a cohesive life history. This study investigates three important links in the chain from spawning adults to the next generation: 1. Where do adult fish spawn? 2. Where do the juveniles spend their first year of life? and, 3. What is their health and condition?

To find spawning areas, ultrasonic transmitters were attached to gravid females during spawning season. The areas where fish were most often detected were sampled with an epibenthic sled to collect fertilized eggs as evidence of spawning activity. Results indicated that spawning most likely occurs in the Lighthouse Point-Morris Cove area, and perhaps at the southern end of Long Wharf. Recaptures of tagged fish after the spawning season came from as far away as the Charlestown Breachway in Rhode Island and the area south of Fisher's Island (NY), demonstrating that fish spawning in New Haven Harbor contribute to fisheries as far away as Block Island Sound.

The second and third questions were addressed by conducting periodic beam-trawls to compare the relative numbers and health of juveniles in four different locations in the harbor-river system. These included Morris Cove, a shallow, sandy embayment on the eastern side of the harbor; the Long Wharf area, a mud flat west of the main channel and north of the Morris Cove site; the Reach site, located in the Grand Avenue Bridge Reach section of the Quinnipiac River; and the Hemingway Creek site, located further upstream where Hemingway Creek empties into the Quinnipiac River.

In the spring, more young-of-the-year fish were caught at Long Wharf than at Morris Cove, where one-year-old fish were more common. The fish at Hemingway Creek in the Quinnipiac River were smaller, less abundant, and had a higher incidence of fin rot than fish caught in other locations. The overall prevalence of axial skeletal anomalies in the young fish is similar to the prevalence in adult fish. The prevalence of axial skeletal anomalies in the abdominal region of young fish is higher than in the adults suggesting that this type of anomaly may be fatal to young fish.

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Habitat Models for Juvenile Pleuronectids Around Kodiak Island, USA

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Juveniles of four species of Pleuronectid flatfishes were abundant in bays and nearshore areas around Kodiak Island, Alaska during August 1991 and August 1992. Linear discriminant analyses were used to identify physical characteristics important in determining presence or absence of juveniles for each species in 1991 and resulting discriminant functions were tested on 1992 data. Discriminant functions predicted presence more accurately than absence in 1991 for rock sole (*Pleuronectes bilineatus*), yellowfin sole (*Pleuronectes asper*), and Pacific halibut (*Hippoglossus stenolepis*). In 1992, absence was predicted more accurately than presence for these three species. The result was opposite for flathead sole (*Hippoglossoides elassodon*) in 1991 with absence being more accurately predicted, while in 1992 presence and absence were predicted equally well.

The distribution of flatfishes captured in 1991 were examined with respect to the following conceptual habitat models which had been produced from the mathematical models based on 1991 data.

MODEL #1: Age-0 rock sole are found predominantly in water depths less than 70 m on sand or mixed sand substrate within 10 km of bay mouths.

MODEL #2: Age-0 flathead sole are found predominantly in water depths greater than 70 m on mud or mixed mud substrate throughout bays.

MODEL #3: Age-0 Pacific halibut are found predominantly in water depths less than 70 m on mixed sand substrate near or outside mouths of bays.

MODEL #4: Age-1 yellowfin sole are found predominantly in water depths less than 40 m on mixed substrate at upper reaches of bays.

A Multivariate Model of Winter Flounder Maturation: What's Age got to do with it Anyway?

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Traditional models of fish maturation invoke fish length or age as explanatory variables for the onset of maturation, since these variables are "useful" data with respect to stock assessments and management plans. However, if onset examines the variability in body weight for fish of a given size, particularly over the range of sizes encompassing the critical "window" of first maturation, it is apparent that body weight or perhaps condition factor may explain the maturation process better than length or age.

To test this hypothesis, data for slower-growing, later-maturing Gulf of Maine winter flounder and faster-growing earlier-maturing Georges Bank winter flounder are analyzed. Univariate and multivariate models are constructed to evaluate the relative contribution of length, age, and body weight/condition factor to the one of maturation. Finally, the practicality and applicability of a weight-based maturation model is considered within the framework of assessment and management requirements.

Growth Rates at *Ad Libitum* Rations of Juvenile *Etropus crossotus* and *Citharichthys spilopterus* in North Carolina Coastal Waters

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Preliminary laboratory experiments were conducted at the National Marine Fisheries Service Laboratory in Beaufort, North Carolina to determine temperature-controlled maximum growth rates in two flatfish species. These growth rates will be compared to field rates in different parts of the fish's ranges to test whether food limits growth. From May-August 1994, three 14-day trials were conducted at 15, 23, and 30ºC. Mean absolute growth rates at *ad libitum* rations for *Etropus crossotus* at sizes of 30-40 mm were 0.2 mm/d at 15ºC, 0.30 mm/d at 23ºC, and 0.23 mm/d at 30ºC. Fish with initial lengths of 70-80 mm had growth rates of –0.03 mm/d, 0.28 mm/d, and 0.10 mm/d, respectively. Growth rates of *Citharichthys spilopterus* (65-80 mm) were 0.05 mm/d, 0.11mm/d, and 0.09 mm/d, at the same temperatures, respectively. The patterns of mean and maximum growth showed the expected relation to temperature with a peak at the intermediate temperature (23ºC). However, differences among means were not significant in *Citharichthys spilopterus* and the smaller size class of *Etropus crossotus*, owing to high variability. Upper quartile rates may be better estimates of maximum growth rates. The comparison of laboratory-derived maximum growth rates with field growth rates is discussed as a test implication for the hypothesis that recruitment variability is not controlled by food.

Submersible Observations of Flatfish Nursery Habitats in the New York Bight

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In order to determine the distribution of fish nursery areas in the New York Bight, we conducted visual transects with a 4.7-m submersible at eleven stations along two benthic transects over depths from 15-85 mm during August 2-12, 1994. Based on our preliminary analysis to date, there is a very strong cross-shelf zonation for recently settled flatfishes. The Gulf Steam flounder (*Citharichthys arctifrons*) was most abundant at our deepest stations (65-85 m), while yellowtail flounder (*Pleuronectes ferrugineus*) was most abundant at depths of 35-50 m. The fourspot flounder (*Paralichthys oblongus*) was restricted to our shallowest sites (15-20 m). There was little overlap of these species at any dive site. The size frequency of fish observed varied with the species, but most individuals were 40 mm TL or less, while *Citharichthys* were smaller (<25 mm). The smaller sizes observed corresponded with the maximum reported sizes of the late larval stages of these species, indicating that we were observing recently-settled fishes. These *in situ* observations suggest that a submersible is much more efficient at detecting these recently-settled fishes than are surface-operated beam trawls, because density estimates for the latter ranged from 1-8% of the total observed from the submersible. In summary, we believe these are the first data to document the distribution of nursery areas for these species, and this technique, with some modifications, offers some distinct opportunities for defining nursery areas and providing insights into recruitment processes on the continental shelf.

Biodiversity and Distributional Patterns of Symphurine Tonguefishes (*Symphurus*: Cynoglossidae: Pleuronectiformes) Occurring in the Western Atlantic Ocean

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Including two new species, 24 of 72 species now recognized in *Symphurus* occur in the western Atlantic Ocean. *Symphurus* is the most diverse flatfish taxon occurring in this region. Diversity of species recorded in this area is comparable with that in the Indo-Pacific (n=24), and is greater than that (n=17) occurring in eastern Pacific or eastern Atlantic (n=6) regions. High diversity of *Symphurus* species in the western Atlantic reflects the presence there of species groups that are highly successful in exploiting shallow-water environments, which are more diverse in the western Atlantic than in other regions of the Atlantic and eastern Pacific Oceans. Although *Symphurus* species occur from Nova Scotia (ca. 45ºN) to Argentina (ca. 38ºS), this genus is primarily a tropical and warm-temperate taxon. All 24 western Atlantic *Symphurus* occur between 34ºN and 25ºS latitudes, with the most diverse assemblage of species (12 species) found in tropical regions. Geographical distributions of individual species are influenced by evolutionary relationships and by major oceanographic features. Faunal boundaries traditionally recognized for other groups of marine organisms also separate different components of this taxon geographically. Few species transcend these boundaries successfully. Ecological distributions are strongly influenced by depth and substrate distributions within the geographic range.

Hudson/Raritan Estuary Winter Flounder Studies

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All fishery surveys of the lower Hudson and Raritan Bays and tributaries that included information on winter flounder are reviewed. Early data on distribution and abundance are compared with results of recent benthic finfish surveys with respect to habitat utilization. Depth zones and bottom type are related to the seasonal abundance of various size groups of winter flounder.

Salinity-related Changes in Resource Use by Two Sympatric Pleuronectid Flounders in Great Bay Estuary, New Hampshire

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Smooth, *Pleuronectes putnami*, and winter, *P. americanus*, flounder are morphologically similar and sympatric from Newfoundland to Massachusetts. Little is known about the interaction between these two species. The purpose of this study was to compare the use of estuarine resources by these two flounder species in Great Bay Estuary, New Hampshire. The species partially segregated along a salinity gradient, with smooth flounder occurring more abundantly at lower salinity stations than winter flounder. However, the species distributions were very dynamic and the amount of habitat use overlapped, and therefore the potential for interactions between the species, varied seasonally in response to varying salinity. Growth experiments indicated that physiological constraints played a major role in determining species distributions. Both species preyed on a variety of benthic invertebrates and exhibited a high overlap in prey usage. Cluster analysis produced groupings based on habitat type and body size rather than species. Changes in food habitats were associated with changes in abundance of prey items. Little evidence of prey switching or changes in niche breadth were associated with increasing overlap in habitat use, suggesting that direct competition for food was not important between these species.

Ontogeny of Functional Morphology of Feeding in Larval Fish: Winter Flounder *versus* Atlantic Cod

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Abstract not available

Abstracts
Poster Presentations

Secretion of 2, 4-Dichlorophenoyyacetic Acid (2, 4-D) by Renal Proximal Tubule Primary Cultures is Inhibited by Phorbol Ester*

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The fate of winter flounder in polluted waters depends in part on their ability to secret toxic organic anionic pollutants. Factors that influence this system can therefore influence survival. To date, regulators of this transporter are uncertain. Because organic anion secretion is strongly expressed in primary monolayer cultures of winter flounder proximal tubule, this tissue was used to characterize the renal transport of herbicide. Tissues were mounted in Ussing chambers and short-circuited. The herbicide 2, 4-dichlorophenoxy acetic acid (2, 4-D) is normally actively secreted by the flounder kidney. Transepithelial transport of ^{14}C-2, 4-D (10μM) by control tissues revealed net secretion of 0.55 ± 0.11 nmoles/cm^2/h (secretory flux: 0.67 ± 0.05; reabsorptive flux: 0.12 ± 0.03). Probenecid (1mM) significantly ($P < 0.05$) inhibited secretory flux (0.51 ± 0.09) but not reabsorptive (0.19 ± 0.06), yielding reduced net secretion of 0.32 ± 0.14. HPLC analysis demonstrated that 2, 4-D was secreted intact by this system. To determine if there was regulation through intracellular Ca^{2+} was raised by the ionophore A23187 (2μM) and the Ca-ATPase inhibitor, thapsigargin (0.2 μM). Neither had any effect on 2, 4-D transport. The adenylate cyclase stimulator, forskolin (10μM) had no effect; however, 1 μM phorbol 12-myristate 13-acetate (PMA) significantly ($P<0.05$) inhibited net 2, 4-D secretion (control: 0.96 ± 0.13; PMA: 0.55 ± 0.04). Because PMA also inhibited phloridzin-sensitive Na-dependent glucose transport, phorbol-12, 13-didecanoate (PE) was tested. With 1 h preincubation, PE significantly ($P<0.05$) inhibited net 2, 4-D secretion (control: 0.75 ± 0.06; PE: 0.53 ± 0.07) and had no effect on glucose transport. Thus, a protein kinase C, insensitive to elevated calcium, may have a role in the regulation of this process. Determining the regulation of organic anion transport will further the understanding of how this animal adapts to pollution.

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*Supported by Connecticut Department of Environmental Protection, Long Island Sound Resource Fund.

Gonadotrophin Induction of Testostrone and Estradiol-17 in Winter Flounder *(Pleuronectes americanus)* Ovarian Follicle Cultures

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Studies on winter flounder reproduction were designed to elucidate causal connections between PCB contamination, endocrine function and fecundity. In support of this effort, development of *in vitro* techniques to assess gonadotropin and steriod hormone production were required. Winter flounder were collected from uncontaminated areas in Narragansett Bay and Long Island Sound by otter trawl. Pituitary extract prepared from winter flounder and two mammalian gonadotropins, ovine-LH and human chorionic gonadotropin, were used to induce testosterone and estradiol-17β production in follicle cultures. Testosterone and estradiol-17β levels were determined in the follicle cultures by radioimmunoassay. In addition to measuring *in vitro* hormone production, the follicle size frequency and the gonadosomatic index were also measured on individual fish. All three gonadotropins significantly induced testosterone production in a dose-dependent fashion. Estradiol production, however, was stimulated only by human chorionic gonadotropin. Stage of ovarian development appeared to contribute to variability observed in testosterone and estradiol-17β levels between individual winter flounder. Further research will incorporate these findings to study whether PCBs affect flounder reproduction.

Grossly Observed Lesions of Flatfish from Various Northeast Embayments

S. A. MacLean[1] and J. J. Evans[2]

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Oxford, MD 21654

Winter flounder (N=919), windowpane flounder (N=404), and fluke (N=30) collected at various estuarine/coastal sites in the northeastern United States were examined grossly for lesion occurrence. Lesion types included ulceration, finrot, nodules/cysts, pigmentation anomalies, hemorrhages, cataracts, and those caused by a variety of parasites. Pigmented cysts caused by metacercariae were by far the most commonly occurring lesions. Fin rot, ulcerations and gill erosion were found predominantly in winter flounder, to a lesser extent in windowpane flounder, and in fish from relatively clean as well as contaminated sites. Fluke collected from Delaware Bay did not have these lesions. Individual fish collected from Boston Harbor tended to have multiple lesions of several types as compared to fish from other sites, which had one or two types of lesions. Results of the study will be discussed relative to the level of site contamination, and age and habits of the fish examined.

Heat Shock Stimulation of Daunomycin Secretion by Flounder Renal Proximal Tubule Cultures

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Department of Physiology and Neurobiology
Storrs, CT 06269-3042

Cells may respond to stressful conditions so that their tolerance of the same or different stressful conditions is increased. This tolerance is manifest as increased cellular survival and (or) tissue function. For example, in cultured renal proximal tubule cells, mild heat or chemical stress stimulates transepithelial sulfate secretion, thereby protecting them from damage by subsequent, more severe heat or chemical stress. If the renal secretion (and therefore excretion) of a toxin were similarly affected by stress, an organism's ability to tolerate it would also be influenced by prior exposure of that organism to stress. To determine whether toxin secretion is responsive to stress, we examined the effect of heat shock on the transepithelial transport of daunomycin (DAU), a P-glycoprotein substrate, by primary monolayer cultures of winter flounder renal proximal tubule epithelium mounted in Ussing chambers under short-circuited conditions. Cultures performed active net secretion of DAU (0.064 ± 0.027 nmol/cm^2/h). Mild heat shock (5ºC elevation for 6-8 h followed by return to normal temperature) almost doubled DAU secretion (0.114 ± 0.026 nmol/cm^2/h). The protein synthesis inhibitor, cycloheximide, inhibited DAU secretion by heat shocked-cultures approximately 40%, indicating that protein synthesis is involved in the stimulation of DAU secretion by heat shock. To elucidate the mechanism of DAU secretion in heat-shocked tissues, we examined the effects of several compounds on DAU secretion. The P-glycoprotein substrates, verapamil, vinblastine, and cyclosporin A, effectively inhibited DAU secretion. The organic cation, tetra-ethylammonium, also inhibited DAU secretion, although its effect was smaller, and tetraethylammonium secretion was inhibited by vinblastine. Finally, DAU secretion was not inhibited by the organic ion, para-aminohippurate, and para-aminohippurate secretion was not inhibited by vinoblastine. None of the treatments affected the transepithelial reabsorptive flux of DAU. There was no nonspecific toxic effect of any inhibitor on the tissues; the transepithelial and the electrical characteristics of the tissues, including rheogenic glucose transport, were unaffected by any of the treatments. Reaction of tissues with a monoclonal antibody to P-glycoprotein (C219) revealed this transporter on only apical microvilli. The data indicate that flounder possess an active mechanism for renal excretion of DAU that is stimulated by mild heat shock. This mechanism is distinct from organic anion, but not organic cation, transport and has characteristics consistent with transport by an apical P-glycoprotein.

Life History Aspects of Blackcheek Tonguefish, *Symphurus plagiusa* (Cynoglossidae: Pleuronectioformes), an Obscure Abundant Flatfish in Chesapeake Bay and the Coastal Waters of Virginia

M. R. Terwilliger

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Gloucester Point, VA 23062

The blackcheek tonguefish, *Symphurus plagiusa*, is a species of shallow water flatfish that ranges from Connecticut southward through the Florida Keys and Bahamas. From Chesapeake Bay and southward it is an abundant estuarine species. This species is the second most abundant pleuronectiform occurring throughout Chesapeake Bay and its tributaries, second only to hogchokers in abundance. Information on life history parameters of this species is limited. Blackcheek tonguefish will be collected primarily via the Virginia Institute of Marine Science's juvenile finfish and blue crab stock assessment monthly trawl surveys of the lower Chesapeake Bay and fixed station mid-channel transects in each of the three major Virginia tributaries: the York, James, and Rappahannock rivers. Each specimen will be measured and weighed; gonads will be excised, examined macroscopically to determine sex and state of maturity, and weighed. Gonads selected for histological examination will be fixed in Davidson's fixative to examine seasonal patterns of development and maturity, while gonads selected for fecundity measurements will be stored in 10% buffered formalin. Sagittal otoliths will be removed from all fish and will be examined for yearly growth rings to determine growth rates, age structure, age at maturity, and number of year classes present within Chesapeake Bay.

Validation of Daily Increment Formation in Juveniles of Four Flatfish Species: *Symphurus plagiusa*, *Scophthalmus aquosus*, *Paralichthys lethostigma* and *Etropus crossotus*

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University of South Carolina
Belle W. Baruch Institute for Marine Biology and Coastal Research
Columbia, SC 29208

Juvenile *Symphurus plagiusa*, *Scophthalmus aquosus*, *Paralichthys lethostigma* and *Etropus crossotus* were collected in the North Inlet area of South Carolina using a 1-m beam trawl. Live specimens were transported to the lab and the otoliths were marked by submerging the fish in alizarin complexone solution (75 ppm for 24 hours). The fish were kept in individual containers and given food *ad lib* for a period between 7 and 21 days. After this period the fish were sacrificed and the otoliths were removed. The sagital otolith of the ocular side of the fish was prepared for increment analysis.

Examination with a UV-microscope and a scanning electron microscope validated daily increment formation in all four species. Daily increment width was variable and is related to diet.

The Anisotropic Structure of Fish Scale Patterns and its Consideration in the Quantification of Seasonal Growth

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Fish scale patterns are divided into seasonal growth zones, with each zone providing information about variability of the fish's growth rate. The data derived from these scales are broadly used in life history studies of fish. In our approach, the information about seasonal growth zones is derived from a plot describing relative widths of the spaces between circuli from the center of the scale to its margin. The shape of this plot is highly dependent on the direction of the measurement chosen due to numerous breaks and confluences of the circuli. Thus, scale patterns are anisotropic objects. Presently, there is no formal procedure for the quantification of seasonal growth zones that takes into account the anisotropic nature of the scale pattern.

The objective of this study is to develop a formal procedure to quantify seasonal growth zones, which incorporates the anisotropic nature of scale patterns. The tool for solving this problem is a N-dimensional model of the scale pattern. This model is based on a table that describes the width of the circuli spacing in N directions and a plot that represents the structure of the circuli such that all breaks and confluences are taken into account.

Plots of the relative widths of the spaces between circuli were plotted for N directions. The plot was used to combine patterns for different directions into a common chart. The resultant chart described the variability of the circuli for an entire scale. A Boolean function was used to define the notion of the "stability of circuli widths" in a context of the variability of the scale structure. This notion was introduced in order to investigate the impact of the antisotropic structure of scale patterns on the accuracy of the quantification of seasonal growth zones and to help in the selection of the version of circuli structure.

Scales of the yellowtail flounder, *Pleuronectes ferrugineus*, of different ages were analyzed. The results showed that the method reduced incertainty in the recognition of seasonal growth zones as well as the quantification of the age of these fish. Further studies to quantify variability in growth patterns between fish collected from different areas, representing potentially different stocks, are underway.

Analysis of Winter Flounder Population Structure Using Random Amplified Polymorphic DNA*

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Winter flounder, a valuable fish to both sport and commercial fishermen, return to natal estuaries along the northeastern seaboard to spawn. If fish returned strictly to their natal waters, slight genetic distinctions between populations would be expected. This characteristic would be expected to be prominent in the offshore population at Georges Bank, where these fish are morphologically different, sporting a darker color and larger size. Using random amplified polymorphic DNA (RAPDs), we examined eleven onshore populations and the offshore population at Georges Bank for genetic variation, particularly looking at those from the greatest geographical distance. Each RAPD banding pattern type was assigned a genotype, and each individual was described by a compiled genotype determined from the pattern of all primers examined. There was as much variation found within populations as between populations; the statistical analysis supports the previous mitochondrial DNA studies that indicate there is no genetic distinctness among any population.

*This research was supported by a Vansant grant, the Biological Research Fund and the Howard Hughes Medical Institute Summer Research Program at Ursinus College, and the Council of Undergraduate Research.

Distribution, Abundance, and Habitat Preference of Juvenile Winter Flounder (*Pleuronectes americanus*) in the Shore Zone of Narragansett Bay, Rhode Island

C. Powell and R. Jones

Rhode Island Division of Fish, Wildlife and Estuarine Resources
Great Swamp Management Area
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West Kingston, RI 02892

Data on juvenile winter flounder have been collected monthly from June through October since 1986 as part of an ongoing juvenile finfish survey of Narragansett Bay. Length frequency, distribution, and abundance data were collected from sixteen stations sampled with a 200-foot beach seine. Trends in abundance and distribution throughout the bay are discussed. Overall, upper bay and Sakonnet River stations had the highest density of juveniles. Mean abundances were higher during the month of July followed by June and August, respectively. Our data indicate that shore-zone juveniles prefer sandy-mud substrate regularly interspersed with macroalgae dominated by *Ulva lactuca*.

The Importance of Microzooplankton in the Diet of Winter Flounder Larvae

S. M. Gallager, L. Davis, and P. Alatalo

Woods Hole Oceanographic Institution, Woods Hole, MA 02543

Abstract not available

Marine Basins as Potential Winter Flounder Habitat

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Clinton, CT 06413

Marina basins are man-made, dredged habitats that are conspicuous features of coastal ecosystems like Long Island Sound. Despite the large volume of information on the importance of finfish nursery grounds, there has been little attention given to the potential importance of marina basins as juvenile habitat. The major purpose of this study was to document the usage of Cedar Island Marina Basin (Clinton, CT) by juvenile winter flounder. Specific objectives were to: (1) evaluate general diversity of juvenile species utilizing the Cedar Island Marina Basin, (2) evaluate seasonal abundance patterns of juvenile winter flounder, and (3) describe feeding habits of juvenile winter flounder in the marina basin.

A one-meter beam trawl survey was conducted at three stations and monitored on a weekly basis (1989-1991) within the Cedar Island Marina Basin. A total of 28 juvenile sized finfish species were collected during the study period. Large numbers of juvenile winter flounder were found to inhabit the marina basin with daily population estimates in some months exceeding 9,000 fish. Lowest numbers of winter flounder were found during the spring and highest numbers during the summer and fall. Density estimates were highest in 1990 and declined at all stations in 1991.

A total of 31 prey species were identified from 92 flounder stomachs examined. The diets of 01-49 mm sized flounder were dominated by the spionid polychaete *Streblospio benedicti* and crab zoea. The diets of 50-99 mm flounder were found to be extremely diverse with over 26 prey species reported. The dominant food source was again *Streblospio benedicti.* A major change occurred in the diets of 100-149 mm flounder with crustaceans increasing in importance and polychaetes decreasing. The dominant prey species was the commensal crab *Pinnixia spp*. Amphipods also increased in importance as a prey group. The trend of increasing importance of crustaceans and declining importance of polychaetes continued into the 150-199 mm size class. The dominant prey species were the hermit crab *Paguras longicarpus* and *Pinnixia spp.*

Identification and Characterization of CytochromeP-450s in Juvenile Winter Flounder

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Storrs, CT 06269

Due to their role in detoxification and their high degree of substrate specificity, cytochrome P-450s have great potential for use as biomarkers. Although previous field studies have demonstrated a relationship between pollutant levels and P450E activity in many fish species, there is still a serious gap in our understanding of P-450 systems in fish. The primary goals of this study were to use biochemical and molecular tools to begin to further characterize the one previously identified fish P-450 and to attempt to uncover other isoforms. Juvenile winter flounder were ideal for this study due to their limited range, lack of sex hormones (known to affect P-450 activity), their potential for exposure to benthic contaminants, and their prevalence in local marine waters. Enzyme activities were determined in liver microsomes using substrates specific to P-450IA1 (Ethoxyresorufin) and 2E1 (Chlorzoxazone). Enzyme activities for both isoforms were above background (both in the range of nmole/mg/min). Assays carried out at different temperatures revealed that the optimum for both 2E1 and IA1 were closely correlated to the environmental temperature. By acclimating fish to warmer temperatures in the laboratory a variation in this pattern was observed and may be indicative of a shift in temperature optimum to correspond with shifting environmental temperatures. Molecular analysis was carried out using a winter flounder genomic library constructed using the cosmid vector pwe-15 with inserts of genomic DNA between 35-40 kb. Primary screen with P^{32} labeled oligonucleotide (49 bases) homologous to a region of rat 2E1 revealed several positive clones. Upon secondary screen three positives were isolated and amplified. DNA from these clones was isolated, digested with restriction enzyme (EcoRI) and analyzed using gel electrophoresis. Banding patterns revealed many high molecular weight fragments. Slot blot and subsequent hybridization to chemiluminescent probe confirmed that these clones contain regions with homology to rat 2E1. In addition PCR of DNA from positive clones using generic P-450 primers resulted in five products ranging in size from 370 bp to 1.8 kb. PCR of genomic DNA using P-450IA1 primers also resulted in five products ranging in size from 300 bp to 1.7 kb. These PCR fragments have subsequently been subcloned and are presently being sequenced.

This study reveals for the first time both molecular and biochemical evidence that a 2E1-like isoform of P-450 exists in fish. Temperature effects observed also emphasize the importance of taking into account environmental and laboratory conditions when utilizing enzyme activities as indicators of exposure to pollutants. Overall it is hoped that by continuing to integrate classical biochemical methods with new genomic analysis tools scientists can acquire a better understanding of how these marker systems are regulated.

A Novel Peptide Isolated from Flounder Skin Has Antimicrobial Properties

G. Diamond and P. Weis

New Jersey Medical School, Newark, NJ 07103

Abstract not available

Maturation of Pleuronectiforms vs. Gadiforms: Size vs. Age

Loretta O'Brien

National Marine Fisheries Service
Northeast Fisheries Science Center
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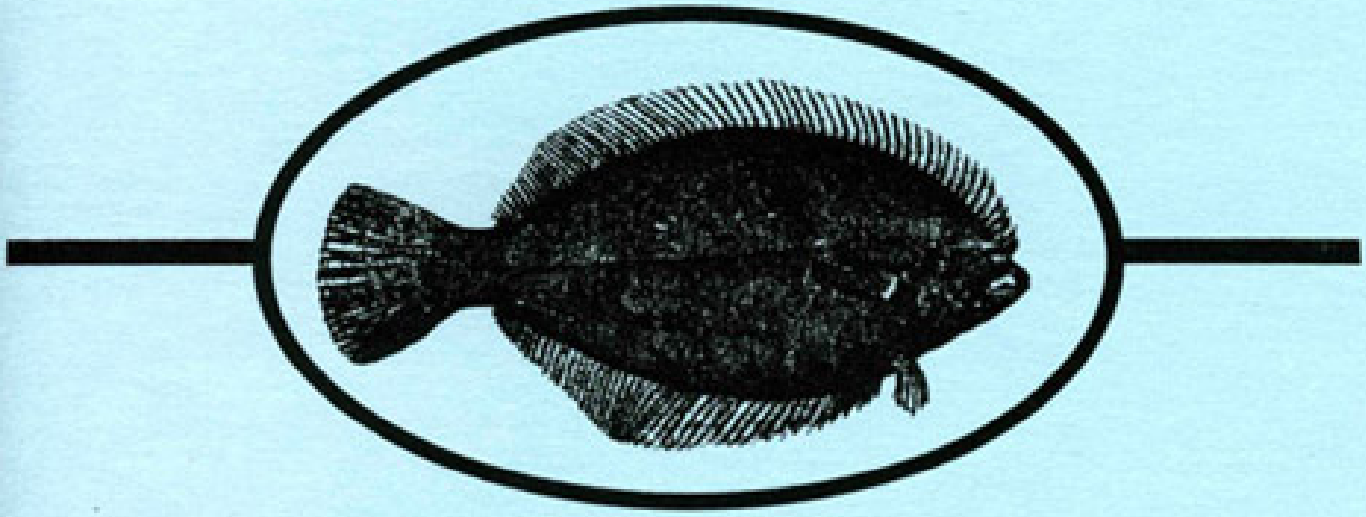
Woods Hole, MA 02543

Observations on maturation stages of several species of pleuronectiformes and gadiforms off the Northeast coast of the USA were analyzed to examine the relationships between fish size or age, and maturity. Maturation schedules and median lengths (L_{50}) and ages (A_{50}) at maturation were derived by fitting the logistic model to the observed proportions. Analyses were generally restricted to observations from 1985-1990 obtained during stratified random bottom trawl surveys conducted in spring and autumn by the Northeast Fisheries Science Center and the commonwealth of Massachusetts Division of Marine Fisheries in waters of the continental shelf from Nova Scotia to Cape Hatteras, north Carolina. Estimates of L_{50} and A_{50} for pleuronectiforms extend over a wide range of ages (1.3 to 4.4 year) within a narrow length span (19.1 to 30.4 cm). Gadiforms exhibit an opposite pattern with estimates of L_{50} extending over a wide range of lengths (22.2 to 41.8 cm) within a relatively narrow age span (1.3 to 2.3). This suggests that maturation is size-dependent for the pleuronectiforms and age-dependent for the gadiforms.

Program and Abstracts

Flatfish Biology Workshop

December 3-4, 1996
Mystic, Connecticut



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Flatfish Biology Workshop
December 3-4, 1996, Mystic, Connecticut

by Conference Steering Committee: Anthony Calabrese (Chair)[1], Allan Beck[2], Jay Burnett[3], Donald Danila[4], Arnold Howe[5], Penelope Howell[6], Ambrose Jearld[3], Chris Powell[7], and Anne Studholme[8]

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Fifth in a series of Flatfish Biology Conferences

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts

February 2008

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National Oceanic and Atmospheric Administration
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Northeast Fisheries Science Center
Woods Hole, MA

Flatfish Biology Workshop

December 3-4, 1996, Best Western Sovereign Hotel, Mystic, Connecticut

Oral Presentations

Tuesday, December 3rd

8:00 a.m. **Registration/Coffee-Best Western**

8:45 a.m. Welcome and Introduction
Anthony Calabrese, Conference Chair
National Marine Fisheries Service
Northeast Fisheries Science Center
Milford, CT

Michael Sissenwine, Directer
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, MA

Session I

Penny Howell, Chair
Connecticut Department of Environmental Protection
Old Lyme, CT

9:00 a.m. Abundance and Distribution of Young-of-the-Year Winter Flounder (*Pleuronectes americanus*) in Point Judith Pond, RI
M. P. O'Connor
University of Rhode Island, Department of Fisheries, Animal and Veterinary Science, Kingston, RI

9:20 a.m. Diel and Tidal Patterns of Distribution of Young-of-the-Year Windowpane Flounder, *Scophthalmus aquosus*
M. J. Newman and K. W. Able
Rutgers University Marine Field Station, Institute of Marine and Coastal Sciences, Tuckerton, NJ

9:40 a.m. Distribution and Abundance of Hogchoker, *Trinectes maculatus*, in Delaware Bay: Preliminary Comparisons between Restored and Reference Marshes
R. O. Bush and K. W. Able
Rutgers University Marine Field Station, Institute of Marine and Coastal Sciences, Tuckerton, NJ

10:00 a.m. Spatial Distribution of Juvenile and Adult American Plaice, *Hippoglossoides platessoides*, in the Georges Bank-Gulf of Maine Region
L. O'Brien
National Marine Fisheries Service, Woods Hole Laboratory, Woods Hole, MA

10:20 a.m. **Coffee Break**

Session II

Chris Powell, Chair
Rhode Island Division of Fish and Wildlife
Kingston, RI

10:40 a.m. Determinants of Depth Selection and Geographic Distribution of American Plaice in the Southern Gulf of St. Lawrence
D. P. Swain and R. Morin
Gulf Fisheries Centre, Department of Fisheries and Oceans, Moncton, New Brunswick, Canada

11:00 a.m. The Role of Estuarine Circulation Patterns in Regulating the Settlement of Juvenile Winter Flounder (*Pleuronectes americanus*) in Coves Near Ocean Inlets
M. C. Curran[1], R. J. Chant[2], K. W. Able[1], and S. M. Glenn[2]
[1]Rutgers University Marine Field Station, Tuckerton, NJ and [2]Institute of Marine and Coastal Sciences, New Brunswick, NJ

11:20 a.m. *Symphurus civitatium* Ginsburg, 1951 (Pleuronectiformes: Cynoglossidae) A Second Estuarine-dependent Tonguefish in Coastal Waters of the Southern United States
T. A. Monroe[1], R. L. Allen[2], D. M. Baltz[3], and S. Ross[4]
[1]National Marine Fisheries Service, National Systematics Laboratory, Washington, DC, [2]Louisiana State University, Department of Oceanography and Coastal Sciences, Baton Rouge, LA, [3]Louisiana State University, Coastal Fisheries Institute, Baton Rouge, LA, and [4]North Carolina National Estuarine Research Reserve, Wilmington, NC

11:40 a.m. Systematics and Distribution of Commercially Important Paralichthyid Flounders in Argentinean-Uruguayan Waters (*Paralichthys, Paralichthyidae*)
J. M. Diaz de Astarloa[1] and T. A. Munroe[2]
[1]Departmento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales Universidad Nacional de Mar del Plata, Argentina and [2]National Marine Fisheries Service, National Systematics Laboratory, Washington, DC

12:00 p.m. **Hosted Lunch**

Session III

Ambrose Jearld, Chair
National Marine Fisheries Service
Woods Hole, MA

1:00 p.m. Ciliated Epithelium in the Developing Digestive Tract of the Larva of the Atlantic Halibut, *Hippoglossus hippoglossus* (Linnaeus, 1758)
C. Morrison
Department of Fisheries and Oceans, Aquaculture Division, Halifax, Nova Scotia, Canada

1:20 p.m. Cytochrome P450 Activity in Livers of Adult Winter Flounder from Boston Harbor and Cape Cod Bay
K. L. Wall[1], K. L. Jessen-Eller[2], and J. Crivello[1]
[1]University of Connecticut, Department of Physiology and Neurobiology, Storrs, CT and [2]University of Connecticut, Department of Marine Sciences, Groton, CT

1:40 p.m. Light Microscopy and Mucus Histochemistry Study of the Developing Digestive Tract in Larval Yellowtail Flounder, *Pleuronectes ferruginea*, from 3-days Post-hatch to Metamorphosis

C. J. Baglole[1, 2], H. M. Murray[3], G. P. Goff[1, 2], and G. M. Wright[1]
[1]University of Prince Edward Island, Atlantic Veterinary College, Department of Anatomy and Physiology, Charlestown, PEI, Canada, [2]Huntsman Marine Sciences Centre, Department of Aquaculture, New Brunswick, Canada, and [3]Memorial University of Newfoundland, Ocean Sciences Centre, St. John's, Newfoundland, Canada

2:00 p.m. Control of Renal Excretion of Anionic Xenobiotics in Winter Flounder, *Pleuronectes americanus*

P. A. Halpin and J. L. Renfro
University of Connecticut, Department of Physiology and Neurobiology, Storrs, CT

2:20 p.m. Thyroid Hormone-salinity Interactions and Their Influences on Metamorphosis and Growth in Larval Summer Flounder

A. M. Schreiber[1], M. McArdle[1], B. Soffientino[1], A. Scribner[1], D. Bengtson[1], and J. Specker[2]
[1]University of Rhode Island, Department of Biological Sciences, Kingston, RI and [2]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI

2:40 p.m. **Cider and Cheese Break**

Session IV

Anne Studholme, Chair
National Marine Fisheries Service
Highlands, NJ

3:00 p.m. Relationships Between Life History Pattern and Recruitment in Flatfishes

C. Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

3:20 p.m. Nursery Habitat Preferences of Juvenile Flatfish on the Continental Shelf of the New York Bight

B. P. Stevens and R. K. Cowen
Marine Sciences Research Center, SUNY at Stony Brook, Stony Brook, NY

3:40 p.m. Midsummer Habitat Correlates and Patterns of Winter Flounder Distribution in the Navesink River and Sandy Hook Bay, New Jersey

J. P. Manderson, L. Stehlik, A. Stoner, J. Vitaliano, A. Bejda, F. Morello, J. Finn, B. Phelan, and S. Fromm
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

4:00 p.m. Habitat Use and Growth Patterns of Young-of-the-Year Winter Flounder in Three Northeastern U.S. Estuaries

B. A. Phelan[1], R. Goldberg[2], J. Pereira[2], P. Clark[2], A. J. Bejda[1], J. T. Finn[1], and S. A. Fromm[1]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [2]National Marine Fisheries Service, Milford Laboratory, Milford, CT

4:40 p.m. Trophic Linkages Between Different Estuarine Habitats and YOY Winter Flounder, as Determined Using Stable Isotope Ratios

S. C. Wainright[1], S. Y. Litvin[1], K. W. Able[1], A. L. Studholme[2], and A. Calabrese[3]
[1]Rutgers University, Institute of Marine and Coastal Sciences, New Brunswick, NJ, [2]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [3]National Marine Fisheries Service, Milford Laboratory, Milford, CT

5:00 p.m. **Poster Set-up**

5:30 p.m. **Hosted Mixer and Poster Session**

Wednesday, December 4th

8:00 a.m. **Registration/Coffee**

Session V

Don Danila, Chair
Northeast Utilities Environmental Laboratory
Waterford, CT

8:30 a.m. A Review of Milford Laboratory Studies on Effects of Contaminated Environments on Reproductive Success of the Winter Flounder, *Pleuronectes americanus*
F. P. Thurberg, D. A. Nelson, and J. J. Pereira
National Marine Fisheries Service, Milford Laboratory, Milford, CT

8:50 a.m. Reproductive Biology of Blackcheek Tonguefish, *Symphurus plagiusa* (Cynoglossidae: Pleuronectiformes), in Chesapeake Bay, Virginia
M. R. Terwilliger
Virginia Institute of Marine Science, College of William and Mary, School of Marine Science, Gloucester Point, VA

9:10 a.m. Annular Growth Patterns, Age, and Longevity in a North Temperate Estuarine Species of Cynoglossid Tonguefish, *Symphurus plagiusa*, with Comparisons of Growth Parameters among Sympatric Pleuronectiformes
M. R. Terwilliger
Virginia Institute of Marine Science, College of William and Mary, School of Marine Science, Gloucester Point, VA

9:30 a.m. Growth and Mortality of Juvenile Winter Flounder in Two New England Estuaries
C, Meise[1], J. S. Collie[2], J. Widman[3], and P. Howell[4]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, [2]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI,[3] National Marine Fisheries Service, Milford Laboratory, Milford, CT, and [4]State of Connecticut Department of Environmental Protection, Marine Headquarters, Old Lyme, CT

9:50 a.m. Associations Between Liver Lesions in Winter Flounder (*Pleuronectes americanus*) and Sediment Chemical Contaminants from Northeast United States Estuaries
S. Chang[1], V. S. Zdanowicz[1], and R. A. Murchelano[2]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [2]National Marine Fisheries Service, Woods Hole Laboratory, Woods Hole, MA

10:10 a.m. **Coffee Break**

Session VI

Allan Beck Chair
Environmental Advantage Group
Prudence Island, RI

10:30 a.m. Affects of Larval Diets and Light Intensity on Growth, Survival and Pigmentation of Southern Flounder
M. R. Denson and T. J. Smith
South Carolina Department of Natural Resources, Charleston, SC

10:50 a.m. Current Status of Atlantic Halibut Culture in the Maritimes
K. G. Waiwood
Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, New Brunswick, Canada

11:10 a.m. Reproduction of American Plaice, *Hippoglossiodes platessoides*, and Turbot, *Reinhardtius hippoglossiodes*, in Newfoundland Waters
D. M. Maddock, R. M. Rideout, and M. P. M. Burton
Memorial University of Newfoundland, Department of Biology and Ocean Sciences Centre, St. John's, Newfoundland, Canada

11:30 a.m. Investigations into the Causes of Early Larval Mortality in Cultured Summer Flounder
D. Alves[1], D. A. Bengtson[2], and J. L. Specker[3]
[1]University of Rhode Island, Department of Biological Science, [2]University of Rhode Island, Department of Fisheries, Animal and Veterinary Science, and [3]University of Rhode Island, Graduate School of Oceanography, Kingston, RI

11:50 a.m. Progress in Controlled Maturation and Spawning of Summer Flounder (*Paralichthys dentatus*) Broodstock
W. O. Watanabe[1], E. P. Ellis[1], S. C. Ellis[1], M. W. Feeley[2], and R. A. Cooper[2]
[1]Caribbean Marine Research Center, Vero Beach, FL, and [2]University of Connecticut, Marine Sciences and Technology Center, Groton, CT

12:10 p.m. Production of Summer Flounder at Great Bay Aquafarms
G. Nardi
Great Bay Aquafarms, Portsmouth, NH

12:30 p.m. **Hosted Lunch**

Adjourn

Poster Session

Tuesday, December 3rd, 5:30 p.m.

Can Scales be Used to Sex Winter Flounder (*Pleuronectes americanus*)
A. J. Bejda and B. A. Phelan
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

Maturity Stages of American Plaice in the Gulf of St. Lawrence
K. Benhalima and R. Morin
Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, New Brunswick, Canada

Witch Flounder (*Glyptocephalus cynoglossus*) Aquaculture
D. Bidwell, W. H. Howell, N. King, E. Fairchild, and A. Tomlinson
University of New Hampshire, Coastal Marine Laboratory, Newcastle, NH

Pleurocidin-An Antimicrobial Peptide from the Skin Secretions of Winter Flounder
A. M. Cole, P. Weis, and G. Diamond
University of Medicine and Dentistry of New Jersey, Department of Anatomy, Cell Biology, and Injury Sciences, Newark, NJ

Effect of Density on the Growth of Summer Flounder, *Paralichthys dentatus*
E. Fairchild, W. H. Howell, A. Tomlinson, and N. King
University of New Hampshire, Coastal Marine Laboratory, Newcastle, NH

Cloning and Sequencing of Winter Flounder Digestive Enzyme Genes
J. W. Gallant and S. E. Douglas
Institute for Marine Biosciences, Halifax, Nova Scotia, Canada

Juvenile Winter Flounder Injected Subcutaneously with Elastomer: Movements and Mortality Estimates in Two Selected Coastal Salt Ponds
C. L. Gray and M. R. Gibson
Rhode Island Fish and Wildlife, Marine Sciences Section, Wickford, RI

Rearing Juvenile Summer Flounder, *Paralichthys dentatus,* in Different Temperature and Salinity Combinations to Enhance Growth for Aquaculture
G. Klein-MacPhee[1] and M. O'Connor[2]
[1]*University of Rhode Island, Graduate School of Oceanography, Narragansett, RI, and*
[2]*University of Rhode Island, Department of Resource Development, College of Fisheries, Aquaculture and Veterinary Science, Kingston, RI*

Ecology of Sheepscot Bay Winter Flounder
R. Langton, S. Sherman, C. Simard, B. Joule, H. Perkins, and S. Chenoweth
Maine Department of Marine Resources, West Boothbay Harbor, ME

Light Intensity and Salinity Effects on Eggs and Yolk-sac Larvae of the Summer Flounder, *Paralichthys dentatus*
W. O. Watanabe[1], M. W. Feeley[2], S. C. Ellis[1], E. P. Ellis[1], and R. A. Cooper[2]
[1]*Caribbean Marine Research Center, Vero Beach, FL and* [2]*University of Connecticut, Marine Science and Technology Center, Groton, CT*

Abstracts
Oral Presentations

Abundance and Distribution of Young-of-the-Year Winter Flounder (*Pleuronectes americanus*) in Point Judith Pond, Rhode Island

M. P. O'Connor

University of Rhode Island
Department of Fisheries, Animal and Veterinary Science
Kingston, RI 02880

Abundance and distribution of young-of-the-year winter flounder was determined July through October 1996 in Point Judith Pond, RI. The flounder were captured using an enclosed quadrat and then marked subcutaneously with Alcian blue dye with the Panjet inoculator. Recaptures occurred throughout the period and were marked again. The reason for this mark recapture study is to observe young-of-the-year winter flounder movements and habitat requirements. Habitat will be described primarily by the distinctive flora and grain size found in the various sites. Abundance will be compared among the different habitats described. Final results will be discussed.

Diel and Tidal Patterns of Distribution of Young-of-the-Year Windowpane Flounder, *Scopthalmus aquosus*

M. J. Neuman and K. W. Able

Rutgers University Marine Field Station
Institute of Marine and Coastal Sciences
800 Great Bay Boulevard
Tuckerton, NJ 08087

We investigated the diel and tidal patterns of estuarine habitat use by young-of-the-year (YOY) windowpane flounder, Scophthalmus aquosus, at a dynamic sandy beach island just inside Little Egg Inlet, New Jersey. We sampled during each tidal stage (mid-ebb, low, mid-flood, high), over a 24-hr period, in July and August 1994 and 1995 at two sites (depth ≤ 1.4 m at mean low water). Windowpane (n=456, 14.0-148.0 mm TL) were collected with a 6.1-m bag seine (3-mm mesh, 192 hauls) and a 1.0-m beam trawl (3-mm mesh, 192 tows). For both years: 1) windowpane abundance was higher in July than in August (1.86 and 0.51 number/tow, respectively); 2) individuals collected in July were smaller than those collected in August (14.0-143.0 and 40.0-148.0 mm TL, respectively); 3) abundance was higher for the seine than for the beam trawl (2.12 and 0.26 number/tow, respectively); and 4) abundance was higher at the site on the west side of the island than at the site on the southwest side of the island (2.15 and 0.22 number/tow, respectively). Despite these differences between gears, months and sampling sites, windowpane abundances were typically higher during low tides than at other tidal stages. Time of day (dawn, day, dusk, night) had no significant effect on abundance. However, in all cases the lowest catches were recorded during the day.

These data provide evidence that YOY windowpane use inlet beaches as nurseries. Among inlet beach habitats, windowpane abundances may vary due to differences in substrate type, flow rate and/or abundance of prey. We believe that the higher catches observed during low tides may be because: 1) windowpane are moving onto the beach where they are vulnerable to our gears; or 2) only at low tide can our sampling cover areas where windowpane are present.

Distribution and Abundance of Hogchoker, *Trinectes maculatus*, in Delaware Bay: Preliminary Comparisons between Restored and Reference Marshes

R. O. Bush and K. W. Able

Rutgers University Marine Field Station
Institute of Marine and Coastal Sciences
800 Great Bay Boulevard
Tuckerton, NJ 08087

As part of an intensive study of marshes in Delaware Bay, we are evaluating the effectiveness of marsh restoration by examining juvenile fish distribution and abundance in a variety of restored and reference marshes. In preliminary studies during 1996 we sampled in intertidal and subtidal habitats with weirs (n=54) and otter trawls (n=561), respectively. Over the salinity range sampled (0-16 ppt), Trinectes maculatus appear most abundant in creeks at intermediate salinities where they were dominated by 50-80 mm TL (range 25-170) individuals that are approximately 1-2 years old, based on a study conducted by Mansueti and Pauly (1956). Curiously, YOY are absent from our samples to date. In one reference creek system (Madhorse Creek, 10-12 ppt range) in which hogchoker were a dominent component of the fish fauna, the distribution varied with habitat type and season. They seldom occurred on the intertidal marsh surface, were slightly more abundant in shallow subtidal creeks <1m MLW, and most abundant in deeper 2-3 m subtidal creeks. Further studies will continue to evaluate the patterns of habitat use with emphasis on YOY.

Spatial Distribution of Juvenile and Adult American Plaice, *Hippoglossoides platessoides*, in the Georges Bank-Gulf of Maine Region

L. O'Brien

National Marine Fisheries Service
Woods Hole Laboratory
166 Water Street, Woods Hole, MA 02543

The influence of depth, bottom temperature, and sediment type on the distribution of American plaice in the Georges Bank-Gulf of Maine region was explored using a generalized additive model (GAM). Length data collected on Northeast Fisheries Science Center autumn bottom trawl surveys during 1963-1995 were disaggregated into inshore and offshore components and four length groups: immature (1-15 cm); primarily immature (16-23 cm); primarily mature (24-34 cm); and fully mature (> 35 cm) fish. A GAM was fit to each length group to detect any changes in preferred habitat with changes in size. Results indicate that juveniles, in general, occupy shallower depths than adults but temperature does not appear to influence this distribution.

Determinants of Depth Selection and Geographic Distribution of American Plaice in the Southern Gulf of St. Lawrence

D. P. Swain and R. Morin

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Moncton, New Brunswick, Canada EOG 2XO

American plaice, a dominant groundfish species in the southern Gulf of St. Lawrence, has varied five fold in abundance since 1971. The stock has occupied the same geographic range in periods of high and low abundance, maintaining highest abundance in the same sectors of the Gulf during both periods. The area of highest plaice density (preferred areas) tended to expand with population size, but similar expansion was not observed into marginal areas less frequented by plaice. These results are contrary to predictions based on optimal foraging theory and contrast with the pattern observed for Atlantic cod in the southern Gulf. Plaice density was strongly related to depth, with peak densities at intermediate depths of 70-90 m. Contrary to the widespread tendency for juvenile fishes to occupy shallower depths, plaice depth selection was similar for all ages (3-12+ yr) and varied little over time or with population abundance. Plaice density was maximum at the same depths for males and females, but the depth distribution of females tended to be more spread out than males. Females also had a broader geographic range than males of the same age or size. The broad geographic range and depth selection of female plaice, relative to males, are consistent with higher growth rates for females and may reflect a higher foraging rate and more competitive interactions.

The Role of Estuarine Circulation Patterns in Regulating the Settlement of Juvenile Winter Flounder (*Pleuronectes americanus*) in Coves Near Ocean Inlets

M. C. Curran[1], R. J. Chant[2], K. W. Able[1], and S. M. Glenn[2]

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New Brunswick, NJ 08903-0231

Prior research has indicated that coves in the vicinity of Little Egg Inlet in southern New Jersey are settlement areas for the winter flounder, *Pleuronectes americanus*. The goal of the present research was to determine whether the spring circulation pattern in Little Egg Harbor supports the advection of larvae into these coves. The survey consisted of repeated transects using an Acoustic Doppler Current Profiler (ADCP) over a 12-hr tidal cycle. Additionally, two S4 current meters were placed in the harbor near the mouth of one cove over a three-week period during flounder settlement (April-May). Our results indicated that there is a strong northward flow of water from the inlet into the western side of the harbor during the flooding current, and, conversely, there is southward flow of water on the eastern side of the harbor during the ebbing current. Therefore, larvae that hatch in the northern portion of Little Egg Harbor may travel down the eastern side of the harbor during ebbing current and be advected into the coves. Based on plankton tows, more winter flounder larvae were found entering the cove on the flooding current than leaving on the ebbing current, supporting the conclusion that they are moving into the cove. Furthermore, the timing of the decline in larval abundance corresponded to the increase in recently settled benthic juveniles collected by beam trawls. The maximum densities of these juveniles occurred in the cove and this number far exceeded values from outside the cove. Our results have important implications for the population dynamics of winter flounder.

Symphurus civitatium Ginsburg, 1951 (Pleuronectiformes: Cynoglossidae) A Second Estuarine-dependent Tonguefish in Coastal Waters of the Southern United States

T. A. Monroe[1], R. L. Allen[2], D. M. Baltz[3], and S. Ross[4]

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Two tonguefish species, Symphurus plagiusa (Linnaeus) and S. civitatium Ginsburg occur sympatrically in coastal shallow-water habitats in the southeastern United States (including North Carolina and Louisiana estuaries). Approximately 2% of 400 juvenile tonguefishes from North Carolina estuaries were S. civitatium, while in Barataria Bay, LA, this species was very abundant and cosmopolitan in distribution comprising nearly 65% of 3600+ tonguefishes collected with a small beam trawl. Historical literature has regarded S. plagiusa as the only tonguefish species inhabiting inshore habitats in these regions. Presence of a second Symphurus species in estuarine regions has been overlooked most probably due to difficulties in identifying post-settlement and early juvenile stages of these species. Early life history stages of both species, although similar in morphology and with overlapping finray counts, can readily be recognized by differences in caudal-fin rays (10 in S. plaguisa and 12 in S. civitatium), especially when used in combination with other characters including pigmentation patterns, jaw position relative to lower eye position, and morphometric features. Occurrence of two sympatric species of tonguefishes in estuarine environments in the central Gulf of Mexico and southeastern regions of the U.S. potentially compromises results of earlier ecological and distributional studies that assumed presence of only a single tonguefish species in these habitats.

Systematics and Distribution of Commercially Important Paralichthyid Flounders in Argentinean-Uruguayan Waters (*Paralichthys, Paralichthyidae*)

J. M. Diaz de Astarloa[1] and T. A. Monroe[2]

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Species of *Paralichthys* Girard, 1858 are the most valuable flatfishes in demersal fisheries of Argentinean and Uruguayan waters. Recent commercial catches increased from 3,000 T in 1984 up to 11,000 T in 1995, representing more than $65 million in export income for 1995. Although their commercial importance has long been recognized, paralichthyid flounders in this region are not well studied either from a systematic or ecological view point. Six nominal species are reported from Argentinean and Uruguayan waters: *P. bicyclophorus* Ribeiro, 1915, *P. brasiliensis* Ranzani, 1840, *P. isosceles* Jordan, 1890, *P. orbignyanus* Valenciennes, 1839, *P. patagonicus*, Jordan, in Jordan & Goss, 1889, and *P. simulans* Lahille, 1939. However, species descriptions are often vague and incomplete, and reported distributions are dubious. *Paralichthys* sp. were collected on the continental shelf and in estuaries in the south Atlantic between 34-55ºS. Variations in scale morphology, meristic and morphometric characters, osteology and coloration were used to differentiate among *Paralichthys* species co-occurring in this area. Of six species previously reported from this region, only three, *P. isosceles, P. orbignyanus* and *P. patagonicus*, are valid. *Paralichthys bicycloporus* and *P. simulans* are junior synonyms; while *P. brasiliensis*, although reported from coastal waters of Argentina and Uruguay for many years, does not occur here. *Paralichthys orbignyanus* and *P. patagonicus* inhabit marine and estuarine locations and extend as far south as 43ºS. The former is a shallow-water euryhaline species occurring to about 20 m. In contrast, *P. patagonicus* has higher densities between 34-40 ºS at 80-100 m depth. *Paralichthys isosceles* occurs mainly on the inner continental shelf between 43-45 ºS at 70-100 m depth, and reaches its southern limit at about 47 ºS.

Ciliated Epithelium in the Developing Digestive Tract of the Larva of the Atlantic Halibut, *Hippoglossus hippoglossus* (Linnaeus, 1758)

C. Morrison

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There are two regions of ciliated epithelium in the digestive tract of the halibut larva. The anterior region, present by 5 days post-hatch and still present at 25 days post-hatch, probably moves water through the brachial openings into the oesophagus and on to the midgut until the mouth forms at about 29 days post-hatch. A ciliated region is also present in the occluded posterior hindgut, which joins the excretory duct to form a common duct to the exterior. This posterior ciliated region may help to circulate water in the mid- and hindgut until the anus opens directly to the exterior, at about 25 days post-hatch.

Cytochrome P450 Activity in Livers of Adult Winter Flounder from Boston Harbor and Cape Cod Bay

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In conjunction with the New England Aquarium-sponsored Fish Day, in May of 1995 local fishermen collected adult winter flounder from various sites within Boston Harbor. During a separate collection effort the National Marine Fisheries Service collected adult winter flounder from Cape Cod Bay for use as reference samples. Samples of the liver from each individual were excised and a variety of parameters measured. For all fish sampled cytochrome P450 IA1 activity, as measured by Ethoxyresorufin O-dethylase (EROD), was significantly higher in females than males.

At the temperature optimum for the assay (18ºC) females from Cape Cod Bay had significantly higher EROD activity than Boston Harbor females. However, this difference was not apparent when samples were assayed at the ambient temperature of the water from which fish were captured. Cytochrome P450 2E1-like activity, as measured by Chloroxazone 6-hydroxylation, exhibited an opposite trend, such that females had a significantly lower activity than males. Also, at presumed ambient temperatures females from Cape Cod Bay had lower activities than Boston Harbor females, where as no difference was observed at the temperature optimum. Interestingly, it was observed that % liver lipid and total liver protein were significantly lower in Boston Harbor vs. Cape Cod females. However, no direct correlation could be made between these parameters and either of the P450 activities measured. Although P450 IA1 activity has been correlated to exposure to polycyclic aromatic hydrocarbons and polychlorinated biphenyls in a variety of marine species, these results suggest that enzyme activity alone may not serve as a reliable indicator. Also, this work demonstrates the importance of reporting ambient and assay temperature. In mammals, P450 2E1-like activity has been correlated with bioactivation of a variety of small volatile organic compounds such as acetone, trichloroethylene, and vinyl chloride. Thus, it is noteworthy that activity of this compound could be detected at all in feral fish populations.

At this point no conclusions can be made regarding presence of inducting agents of this enzyme activity. As in mammals, physiological factors such as starvation also play a role in P450 2E1 regulation.

Light Microscopy and Muscus Histochemistry Study of the Developing Digestive Tract in Larval Yellowtail Flounder, *Pleuronectes ferruginea*, from 3-Days Post-hatch to Metamorphosis

C. J. Bagiole[1, 2], H. M. Murray[3], G. P. Goff[1, 2], and G. M. Wright[1]

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Successful feeding of larval yellowtail flounder, Pleuronectes ferruginea, is an essential prerequisite to the aquaculture development of this species. This study examines the major ontogenetic changes occurring in the digestive tract of larval yellowtail flounder between 3 and 46 days following hatch using light microscopy and mucus histochemistry.

The digestive tube of larvae fixed in toto in Bouin's fluid was undifferentiated at 3-days post-hatch. Regional differentiation was observed by 7-days post-hatch and by day 10 was complete. Six distinct regions were defined: the buccal cavity, pharynx, esophagus, post-esophageal swelling (PES), intestine and rectum. Goblet cells were present in the buccal cavity, pharynx, and intestine by day 7. A further distinction by day 29 between zone 1 (anterior) and zone 2 (posterior) of the esophagus was based on an increase in goblet cell numbers in zone 2. The PES was defined as a stomach with the appearance of multicellular glands by day 36. The liver was present by day 3 and the pancreas between days 3 and 7. Goblet cells and the apical layer of stratified epithelia lining the buccal cavity, pharynx, and esophagus were strongly positive for acid mucins. The epithelia of the PES/stomach stained weakly for neutral mucins. No mucin staining was associated with the gastric glandular epithelium. The brush border of the intestine and rectum stained strongly for a combination of neutral and acid mucins.

Future work includes lectin-binding studies to determine structural differentiation and enzyme histochemistry to evaluate digestive enzyme activity and distribution.

Control of Renal Excretion of Anionic Xenobiotics in Winter Flounder, *Pleuronectes americanus**

P. A. Halpin and J. L. Renfro

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Department of Physiology and Neurobiology
Storrs, CT 06269

The rate of excretion of toxic pollutants such as the herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) can determine the dwell time within the body and thus potential toxicity. Factors which increase or decrease the rate of pollutant excretion may impact significantly the animal's health. Prior studies had shown that monolayer cultures of winter flounder renal proximal tubule cells (PTCs) were capable of active transepithelial transport of 2, 4-D. In addition, factors such as phorbol esters, which stimulate protein kinase C (PKC), inhibited 2, 4-D secretion, indicating that intracellular signaling systems could modulate secretion. The renal clearance of organic anions is known to vary *in vivo.*

To assess possible regulatory control, we mounted PTCs in Ussing chambers and examined the unidirectional fluxes of ^{14}C-2, 4-D. Test of potential primary messengers of the PKC effect showed that 1 µM dopamine, from the peritubular side only, significantly ($P<0.05$) inhibited net 2, 4-D secretion (control: 1.03 ± 0.07; dopamine: 0.89 ± 0.07). Others have shown that dopamine partially deactivates Na,K-ATPase and –adrenergic agonists stimulate Na,K-ATPase activity in proximal tubule. In PTCs, we found the –adrenergic agonist, oxymetazoline (10µM), transiently, but significantly ($P<0.05$), stimulated 2, 4-D secretion (142% of control).

To examine involvement of the sodium gradient, tissues were preincubated 1 hr in 10 µM 2, 4-D with Na^+-free medium on the luminal side only. After 1 hr, Na^+ was restored to the lumen. Both Na-dependent glucose transport and 2, 4-D secretion were stimulated by this transient increase in the plasma membrane Na^+ gradient. The data indicate that catecholamine may greatly influence rates of pollutant excretion in flounder.

*Supported by NSF

Thyroid Hormone-salinity Interactions and Their Influences on Metamorphosis and Growth in Larval Summer Flounder

A. M. Schreiber[1], M. McArdle[1], B. Soffientino[1], A. Scribner[1], D. Bengtson[1], and J. Specker[2]

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Summer flounder metamorphosis is characterized in part by the migration of the right eye to the left side of the head, a process which permits classification of different larval stages. Thyroid hormones (THs) are believed to directly mediate metamorphosis in all flatfish species, though this hypothesis has only been confirmed for the Japanese flounder. In this study we show that 1) THs are both necessary and sufficient for metamorphosis in summer flounder, 2) a natural TH surge during metamorphic climax is accompanied by an increased growth rate; the chemical inhibition of this surge inhibits growth, though TH supplementation does not enhance growth, 3) larval osmoregulatory tolerances fluctuate during the progression of metamorphosis, 4) salinity interacts with the thyroid axis in a stage-dependent manner, and 5) salinity influences development and growth. The effect of salinity on development and growth may be mediated through an interaction with the thyroid axis.

Relationships Between Life History Pattern and Recruitment in Flatfishes

C. Chambers

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Flatfishes are one group for which data on life history and population characteristics are fairly extensive. I have evaluated data from the literature and other historical sources for the purpose of establishing relationships between life history traits and various measures of recruitment and stock dynamics of flatfishes. Variables of particular interest included features of the early life history (*e.g.*, sizes of eggs and larvae, sizes at settlement or metamorphosis, larval period duration) and those depicting variability in yearclass strength (*e.g.*, CV of abundance of yearclasses). The patterns revealed by the analysis and their implications will be presented, as will suggestions for further work. Among other benefits, this kind of exploratory analysis has utility for establishing a broader context from which species-level investigations on recruitment processes can proceed.

Nursery Habitat Preferences of Juvenile Flatfish on the Continental Shelf of the New York Bight

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Nearshore environments such as estuaries have long been given particular emphasis in the study of fish nursery habitats. Some fish species, however, utilize offshore regions such as continental shelves as their nursery habitat, yet little work has been directed at their habitat requirements. To examine potential offshore nursery areas for juvenile flatfish, cruises in the New York Bight were conducted monthly, between June 1996 to October 1996. A modified 2-m beam trawl was utilized across three transects of seven stations each ranging in depth from 20 to 100 meters. Abundances and distributions of several species including yellowtail flounder, *Limanda ferruginea*, and Gulf Stream flounder, *Citharichthys arctifrons*, indicate relatively discrete juvenile species assemblages across the continental shelf. Furthermore, changes in patterns of distribution for several species during the course of the study indicate possible immigration/emigration and differential mortality processes among sites. These data will be discussed with respect to nursery habitat preferences for offshore flatfish species of the New York Bight's continental shelf.

Midsummer Habitat Correlates and Patterns of Winter Flounder Distribution in the Navesink River and Sandy Hook Bay, New Jersey

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An exploratory survey of the Navesink River and Sandy Hook Bay, New Jersey was performed to begin to examine the influence of landscape pattern on estuarine community interactions. Survey collections were made in late July and early August with beam and otter trawls at 89 fixed stations. Stations were located in a variety of habitats throughout the estuary, including those considered important to winter flounder. A preliminary multivariate analysis was performed to identify the dominant species assemblages, species associations, and important ecological gradients in the system.

Winter flounder (N=196, 33-305 mm total length (TL)) ranked 4th in total abundance among fish species collected and over 90% of the individuals were young-of-the-year (33-117 mm TL). Young-of-the-year flounder were relatively abundant in a variety of shallow water habitats throughout the study area, including marsh creeks and marsh-fringed coves, sandbars, and in habitats characterized by dense aggregations of the amphipod *Ampelisca abdita*. The species frequently co-occurred with the blue crab *Callinectes sapidus* and fluke *Paralichthys dentatus*, which are potentially important predators.

Habitat Use and Growth Patterns of Young-of-the Year Winter Flounder in Three Northeastern U. S. Estuaries

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A comparative study was designed to assess the quality of selected nursery habitats for young-of-the-year (YOY) winter flounder (*Pleuronectes americanus*) in Connecticut and New Jersey. Habitat-specific abundance, distribution, and growth were quantified in common nursery habitats. Habitat types selected for study were characterized by unattached macroalgae (*Ulva lactuca*), eelgrass (*Zostera marina*), and adjacent unvegetated areas and marsh creeks. Habitat-use patterns were determined quantitatively using a 1-meter beam trawl. Young-of-the-year winter flounder utilize primary settlement areas and disperse from them to a variety of vegetated and unvegetated habitats. Eelgrass habitat and adjacent unvegetated areas supported a greater number of YOY than other habitats. Growth was quantified by measuring increases in length and weight of fish held in short-term caging experiments at specific habitat types. Survival and growth varied with habitat type. The greatest growth rates occurred for the smallest individuals and rapidly diminished with time. Comparisons among habitats indicated growth to be similar except for marsh creeks, which were lower. In salt marsh creeks where dissolved oxygen levels were often very low, survival was poor.

These data suggest that eelgrass, macroalgae, and adjacent unvegetated areas are important as nurseries for winter flounder expecially when environmental conditions (*i.e.*, dissolved oxygen and water temperatures) are favorable. Concurrent interpretive studies on stable isotope ratios, RNA/protein levels, and sediment electrochemistry, support conclusions drawn from caging experiments.

Trophic Linkages Between Estuarine Habitats and YOY Winter Flounder, as Determined Using Stable Isotope Ratios

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During the past three years, under the auspices of NOAA's Coastal Ocean Program, we have been conducting studies on habitat utilization and habitat-specific growth in juvenile winter flounder and tautog. These studies have been conducted in three northeastern estuaries (Great Bay/Little Egg Harbor, Long Island Sound, and the Hudson/Raritan), chosen so as to represent a gradient of anthropogenic influence. At a previous workshop, we presented preliminary evidence from the 1st year, showing that YOY winter flounder which were confined to cages within certain habitats *(e.g.,* eelgrass beds) may assume distinctive stable isotopic compositions. We confirmed this pattern with additional caging experiments conducted during the past 2 years. Similar experiments using juvenile tautog indicated that habitat-specific patterns exist for this species as well. In addition to the habitat-specific isotopic compositions within estuaries, we have seen a recurring pattern of higher nitrogen isotope values in estuaries with more human influence, *i.e.*, the Hudson-Raritan and Long Island Sound estuaries. Taken together, our studies indicate that nitrogen isotope ratios may be an indicator of anthropogenic stress in aquatic ecosystems, and that carbon and nitrogen stable isotopes may be used to elucidate patterns of habitat utilization by at least some juvenile fishes within estuaries.

A Review of Milford Laboratory Studies on Effects of Contaminated Environments on Reproductive Success of the Winter Flounder, *Pleuronectes americanus*

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A series of studies on effects of contaminated habitats on reproductive success of the winter flounder have been conducted at the Milford Laboratory in recent years. Flounder collected from polluted urban harbors and from relatively uncontaminated coastal areas were spawned in the laboratory and the resulting eggs and larvae were evaluated for hatching success and larval development. Reduced hatch and impaired larval development were found in fish from contaminated urban harbors when the eggs and larvae were compared to those obtained from flounder taken from cleaner habitats.

Harbor dredging has been suspected as an impediment to flounder recruitment when dredging takes place during the spawning season. When dredging conditions were simulated in the laboratory, reduced hatching success was noted in winter flounder eggs. Both clean and contaminated dredge spoils were used in these simulations. Preliminary results show that although the greatest reduction in hatching success was found with contaminated sediments, both sediments cause some impairment indicating that factors other than chemical contamination may be involved.

The role of a contaminated habitat on the production of the egg-yolk precursor protein vitellogenin was also studied in winter flounder and the results obtained demonstrate contaminant-induced changes in vitellogenin production and transport in these fish.

Reproductive Biology of Blackcheek Tonguefish, *Symphurus plagiusa* (Cynoglossidae: Pleuronectiformes), in Chesapeake Bay, Virginia

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The blackcheek tonguefish**,** *Symphurus plagiusa,* is the only member of the pleuronectiform family Cynoglossidae occurring in Chesapeake Bay. From Chesapeake Bay and southward it is an abundant component of the fish fauna occurring in estuaries and coastal embayments. Despite its relative abundance in different estuarine and marine habitats of the northwest Atlantic, information on the life history parameters of the species is limited. The reproductive biology of *S. plagiusa* was studied of specimens collected via the Virginia Institute of Marine Science (VIMS) juvenile finfish and blue crab stock assessment programs trawl survey of the lower Chesapeake Bay and the three major Virginia tributaries: the York, James and Rappahannock Rivers. Among 556 specimens examined in this study, sex ratio was 223 males (36-190 mm TL) to 343 females (46-202 mm TL), or 1:1.54. Macroscopic inspection of gonadal tissue showed that this species exhibits a protracted spawning period from late May to September. Histological examination of ovarian tissue reinforces these findings. Size frequency distributions of oocytes show no hiatus between oocyte size classes, indicating the *S. plagiusa* is an indeterminate spawner. Based upon reproductive stages of females, it is estimated that *S. plagiusa* matures by age one. Batch and total fecundity estimates will be completed in the near future. The reproductive parameters of blackcheek tonguefish will be compared to those of five sympatric Pleuronectiformes.

Annular Growth Patterns, Age and Longevity in a North Temperate Estuarine Species of Cynoglossid Tonguefish, *Symphurus plagiusa*, with Comparisons of Growth Parameters among Sympatric Pleuronectiformes

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Blackcheek tonguefish, *Symphurus plagiusa*, is the only cynoglossid flatfish occurring in abundance in shallow estuaries and coastal embayments in north temperate latitudes. In Chesapeake Bay, it ranks sixth in abundance in juvenile finfish surveys. Transverse sections of 566 sagittal otoliths were read for ageing. Growth marks are formed once yearly in June. The first annulus is formed at a size range of 88-138 mm TL. Macroscopic staging of ovaries revealed that blackcheek tonguefish reached sexual maturity between 80-130 mm TL. Length at 50% maturity was 97 mm TL. Von Bertalanffy growth parameters were similar for both sexes; Von Bertalanffy equations were: males: $L_t = 196.5\ (1-e^{-.2853(t+.9195)})$; females: $L_t = 190.6\ (1-e^{-.3205(t+.7842)})$. Growth is rapid during the first year for both sexes, then slows rapidly thereafter. Maximum age for both sexes was 5 years. This study revealed overlap in total length between age groups, thus rendering age interpretations based on length frequency unreliable. Detailed growth studies for other cynoglossid species are unavailable; no comparisons could be made among other members of this family. Growth parameters of blackcheek tonguefish were compared to those of five sympatric Pleuronectiformes. A variety of growth schemes are exhibited by these Pleuronectiformes, indicating that several growth patterns exist in fish that successfully endure the rigors of dynamic estuarine environments.

Growth and Mortality of Juvenile Winter Flounder in Two New England Estuaries

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We compared young-of-the-year winter flounder (*Pleuronectes americanus*) populations from a contaminated site, New Haven Harbor, and a clean site, the Connecticut River estuary. Juvenile winter flounder were collected with a 1-m beam trawl at bi-monthly intervals during 1993. This analysis is limited to 3- to 15-cm winter flounder collected from June to October. We fit a length-based model to the observed size-frequency distributions. The mode incorporates the von Bertalanffy growth equation and a mortality rate that decreases exponentially with size. A bootstrap algorithm was used to generate confidence intervals for the growth and mortality coefficients. Growth and mortality rates were significantly higher at the contaminated site than at the clean site.

Associations Between Liver Lesions in Winter Flounder (*Pleuronectes americanus*) and Sediment Chemical Contaminants from Northeast United States Estuaries

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Neoplastic diseases, particularly of bottom-dwelling fishes, are more prevalent in coastal areas than in areas that are relatively pristine. Although sediments in many urbanized estuaries contain high concentrations of contaminants, there is no conclusive evidence linking a specific organic or inorganic chemical to a particular liver lesion in winter flounder (Pleuronectes americanus). Between 1984 and 1986, sediments and winter flounder were collected from 10 sites in the northeast United States ranging from grossly polluted to relatively unimpacted. Sediments were analyzed for polycyclic aromatic hydrocarbons (PAHs), chlorinated pesticides, polychlorinated biphenyls (PCBs), and metals. Gross and microscopic pathological examinations were conducted on winter flounder liver sections. Factor and canonical correlation analysis were used to explore associations between biological and chemical measurements.

In general, inflammatory liver lesions (hepatitis, cholangitis, phlebitis and macrophage aggregate hyperplasia) showed strong positive associations with low molecular weight, petroleum-derived PAHs, tri- to hexa-chlorobiphenyls and chromium, lead, thallium and selenium, but were negatively associated with DDT-type pesticides. Pre/neoplastic lesions (cytoplasmic hepatocellular vacuolation, cytoplasmic bile duct vacuolation and preneoplasia) showed strong positive associations with most PAHs measured, whether petroleum- or combustion-derived, the pesticides dieldrin, transnonachlor and alpha-chlordane, and silver, copper, antimony and tin, but no associations with PCBs were found. Necrotic lesions (necrosis, cell necrosis and hemorrhagic necrosis) showed strong positive associations with hepta- to nona-chlorobiphenyls and arsenic, zinc, nickel, and mercury, and strong negative associations with high molecular weight, combustion-derived PAHs and DDT metabolites.

Effects of Larval Diets and Light Intensity on Growth, Survival, and Pigmentation of Southern Flounder

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Interest in the culture of flatfishes has increased in recent years due to the high consumer demand for these fishes. The southern flounder, Paralichthys lethostigma, is the focus of important commercial and recreational fishes in the south Atlantic and Gulf of Mexico. However, culture requirements of this species are poorly understood.

In 1996, two larval rearing studies were conducted with the southern flounder to examine the effects three larval diets and high and low light conditions have on survival, growth and pigmentation. At 24ºC, five-day old larvae (1.98 ± 0.1 mm TL) initiated feeding on the rotifer Brachionus plicatilis and completed metamorphosis by day 30. In treatment 1 larvae (6 days post-hatch (dph) were fed rotifers (10/ml) days 6-15 and Artemia nauplii (3/ml) day 7 through metamorphosis. The second treatment was fed rotifers from 6 dph through metamorphosis and Artemia 7 dph through metamorphosis, while treatment 3 was fed using the same protocol as treatment 1 plus a commercially prepared larval diet.

Metamorphosis began on day 23 (8.2 ± 0.6 mm TL) and was completed one week later in both studies. No differences in size (11.4 ± 1.2 mm TL), survival (37.5 ± 15.6) or percent natural pigmentation (30.5 ± 12.7) were detected. Treatment 1 was also evaluated in low light (42-387 lx) and high light (801-1820 lx) conditions. Similarly, no differences were detected among treatments reared under low light conditions. No significant change in pigmentation patterns was detected 1 week after metamorphosis.

Current Status of Atlantic Halibut Culture in the Maritimes

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Research on Atlantic halibut has been ongoing at the St. Andrews Biological Station since 1990. Several studies, using wild-caught juveniles, have been undertaken to determine the suitability of the Bay of Fundy area for outgrowing. However, the majority of research effort has focused on solving problems related to the mass production of juveniles. One major problem has been poor survival in the yolk-sac stage which, in this species, is up to 45 days long. This presentation describes methods used to improve survival and larval quality using salinity manipulation. In 1996, the first commercial production of Atlantic halibut juveniles was achieved through a DFO/industry partnership. A description of the commercial rearing techniques used and the problems encountered is presented.

Reproduction of American Plaice, *Hippoglossoides platessoides*, and Turbot, *Reinhardtius hippoglossoides*, in Newfoundland Waters

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Maximizing reproductive output within the somatic constraints imposed by relatively small body cavity in plaice and turbot is likely accomplished through a batch-spawning strategy. Evidence of production and release of distinct batches of oocytes is given for wild American plaice and a similar strategy is suspected for turbot based on initial investigation. Increase in white muscle moisture during the spawning season, observed in American plaice, may indicate the need to draw on protein reserves to push oocytes through vitellogenesis to ovulation. The increase in "jellied" turbot in commercial catches also corresponds to the summer breeding season and represents protein utilization probably routed into production of oocyte batches. There is some evidence that plaice may forego reproductive development in the face of energetic constraints.

Investigation into the Causes of Early Larval Mortality in Cultured Summer Flounder

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Culture of larval marine fishes has long been a problem. In order to increase hatchery production, for either commercial aquaculture or stock enhancement, the cause of mortality in the early larval period, especially during the critical period of first feeding, must be overcome. This experiment was designed to investigate the first-time feeding period in summer flounder (*Paralichthys dentatus*), specifically to determine to what degree mortality is caused by the failure of larvae to initiate feeding. Larvae from individual male x female crosses were cultured in replicate bowls. Mortality rates of larvae in bowls that received food (live rotifers) were compared to those in bowls that did not. In addition, larvae were subsampled from each bowl each day to determine percentage of larvae with food in the gut. Larvae from one cross were also subjected to an experiment in which some of the replicate bowls contained "green water" (algae added) and some contained "clear water" (no algae) (All other experiments were conducted using clear water). Mortality was not due to inability of the larvae to initiate feeding. Large-scale mortality in fed replicates was observed often prior to mortality of unfed control fish and daily subsamples of fed larvae indicated that a high percentage had food in the gut regardless of mortality rate. Inter-replicate variability (*i.e.*, among fed bowls from a single parent cross) was extremely high. Results of the green-water vs. clear-water experiment demonstrated significant differences in growth, but not survival. Our results, especially inter-replicate variability, suggest that something in the rearing environment (differing from bowl to bowl) was responsible for the early larval mortality.

Progress in Controlled Maturation and Spawning of Summer Flounder (*Paralichthys dentatus*) Broodstock

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Wild-caught, adult summer flounder (*P. dentatus*) (n=60, avg. wt.=740 g; range=264-1540 g), collected during September and October 1994 off New Haven, Connecticut and Tuckerton, New Jersey, were transported to Vero Beach, Florida in March 1995 and held in six, 2.6-m^3 fiberglass tanks through November 1995 under three different photothermal regimes: (1) indoor, artificial photothermal conditions simulating natural habitat regimes (natural regime); (2) conditions as in (1) but with seasonal temperatures changes advanced by one month (accelerated regime); and (3) outdoor, ambient conditions in Vero Beach, Florida (ambient regime). Under the natural and accelerated regimes, day length and temperature were held constant after declining to autumnal levels of 10 hr and 17ºC, respectively. Under all treatments, onset of vitellogenesis in females (monitored by biopsy) was associated with declining day length and temperature conditions, with ovarian maturation beginning in the accelerated treatment group, then progressing to the natural and ambient groups.

From 20 September to 28 November 1995, twenty-four hormone-induced spawning trials were conducted with females from the accelerated and natural regimes using a single intramuscular implantation of a cholesterol-cellulose pellet containing LHRH-a (100 mg/kg body wt). Females with initial mean oocyte diameters of 258-456 µm spawned voluntarily 2.5-5.5 days post-implantation, while no response was obtained from females with smaller mean oocyte diameters ranging from 151-231 µm. Two females were spawned twice by LHRH-a pellet implantation. Infrequent, natural spawning without hormone induction was also obtained. Females released from 22.7-396.9 x 10^3 eggs on the first day of spawning, with fertilization rates ranging from 0-93.4% and hatching rates ranging from 0-81.1%. Variable fertilization rates were attributable to inconsistent performance by males.

The results demonstrate that LHRH-a-induced and natural spawning of photothermally-conditioned fish are promising techniques to help meet increasing year-round demands for summer flounder seedstock.

Production of Summer Flounder at Great Bay Aquafarms

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No Abstract available

Abstracts
Poster Presentations

Maturity Stages of American Plaice in the Gulf of St. Lawrence

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American plaice in the Gulf of St. Lawrence, and throughout much of their range, migrate in late autumn to deeper water. The overwintering period that follows involves a cessation of feeding and rapid development of gonads, terminated by an early springtime migration to spawning areas. Groundfish surveys conducted in summer months frequently report difficulty in identifying the maturing reproductive stages, particularly in identifying plaice destined to spawn the following spring. This poster outlines work that we have undertaken to classify maturity stages of plaice throughout the annual cycle, based on morphological and histological observations.

Can Scales be Used to Sex Winter Flounder (*Pleuronectes americanus*)?

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For finfish species such as winter flounder, which exhibit little if any sexual dimorphism, the ability to sex individuals without sacrificing them is problematic. However, the literature suggests that the sexes can be differentiated by the character of the scales on the blind side (*i.e.*, ctenoid for males and cycloid for females). To determine the reliability of this dimorphism, a total of 730 fish were collected from both inshore and offshore locations. Prior to examining the gonads, the blind side of each fish was palpated noting either roughness (males) or smoothness (females). In addition, scales were sampled from 672 fish to ascertain the influence of observer error. Overall only 75% of the identifications were correct, with an error rate of 45% for males and 6% for females. Observer error accounted for 20% of the misidentifications. The effects of utilizing this methodology on estimates of various populations parameters (*e.g.*, sex ratios, size structure) will be discussed.

Witch Flounder (*Glyptocephalus cynoglossus*) Aquaculture

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Although witch flounder (*Glyptocephalus cynoglossus*) consistently fetch the highest prices of any groundfish species landed in New England (larges have averaged $3.80-$5.15/lb thus far in 1996), few, if any, attempts at aquaculture have been made. A lengthy larval period, predicted fragility, and unpredictable timing and location of spawning have been the primary deterrents.

Witch flounder spawn from April through September in the Gulf of Maine. During June and July adult fish were stripped at sea. Milt from several males was used to fertilize the eggs of each ripe and running female. Incubation proceeded in 40 kriesels with stocking densities of approximately 250 eggs per liter and an average water temperature of 7.5ºC. Mean egg diameter was 1.2 mm. Water was changed twice daily through the germ ring stage (due to significant excretion of ammonia during the blastodisc to germ ring transition) and daily thereafter. Fifty percent hatching was reached at an average of 115 degree days. Mean length at hatching was 4.92 mm. At 75% hatching, marine microalgae (*Isochrysis galbana* Tahitian strain) was added to kreisels at a density of 200,000 cells per ml.

Following yolk-sac absorption, larvae were transferred to 200 flow-through tanks. Microalgae was added twice daily at the above density. Mean length at yolk-sac absorption was 6.34 mm. First-feeding larvae were fed a combination of wild zooplankton and cultured rotifers (*Brachionus plicatilius*) from 48-200*i* at a density of 3000 prey per liter. Water temperature averaged 13ºC. Photoperiod was maintained at 17L:7D. Weekly mean increases in length were 19.8%, 11.3% and 29.2% for the three weeks post yolk-sac absorption, respectively. Higher temperatures caused by a problem with a water cooling system may have been responsible for the decline in growth rate during the second week. Witch flounder larvae survived temperatures from 5-19.5ºC, but appeared to grow optimally between 8-12ºC.

Witch flounder have thus far been raised to a maximum length of 47 mm (at this length metamorphosis has not yet occurred) and have been weaned onto a commercial dry diet.

Early attempts at experimentation were unsuccessful due to mechanical problems but future research will examine the following: effect of size and quality of live diets on growth and feeding of first-feeding larvae; effect of temperature on larval growth and mortality; effect of photoperiod on larval feeding and growth; optimal techniques for the collection and maintenance of adult broodstock.

Given current hatching success and larval hardiness, the potential for witch flounder aquaculture on a commercial scale may be quite high.

Pleurocidin -- An Antimicrobial Peptide from the Skin Secretions of Winter Flounder*

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Antimicrobial peptides are found in both myeloid cells and mucosal tissue of a wide variety of species. These peptides are predicted to function as a first-line host defense mechanism against pathogenic organisms. We report the characterization of a novel 25-residue linear antimicrobial peptide, pleurocidin, found in the skin mucous secretions of the winter flounder (*Pleuronectes americanus*).This peptide was purified to homogeneity through multiple chromatographic methods. This purified peptide exhibited antimicrobial activity against *E. coli* in a bacterial cell-lysis plate assay. Mass spectrometry, amino acid sequence analysis, and solid-phase synthesis of the peptide were subsequently performed. Pleurocidin is predicted to assume an amphipathic alpha-helical comformation similar to many other linear antimicrobial peptides. Although there is insignificant homology between pleurocidin and the cecropin, magainin, and defensin families of antimicrobial peptides, there exists a high degree of homology between this peptide and two antimicrobial peptides, ceretotoxin from insects and dermaseptin from amphibians. The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of pleurocidin have been determined against *E. coli*, *Aeromonas salmonicida, Cytophaga aquatilis, Leucothrix mucor, and Pasteurella haemolytica*. This peptide is shown to exert broad spectrum antibacterial activity. Pleurocidin represents the first antimicrobial peptide found in teleost fish and may play an important role in their host defense.

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Effect of Density on the Growth of Summer Flounder, *Paralichthys dentatus*

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Since stocking density can affect growth and survival of cultured finfish, the commercial success of any aquaculture venture depends, in large part, on knowing what the optimal stocking density is. As a rule, increasing density in a tank beyond some upper limit tends to reduce growth and/or survival through mechanisms such as competition for space, increased metabolite production, and cannibalism. This has been shown in several flatfish species (*e.g.*, turbot, *Scophthalmus maximus*; Atlantic halibut, *Hippoglossus hippoglossus*; and Japanese flounder, *Paralichthys olivaceus*). Optimal stocking density is unknown, however, for summer flounder which has become an important commercially cultivated fish in the northeastern United States.

Three different density treatments were employed to determine the optimal stocking density for juvenile summer flounder. Density was measured as percentage coverage of the substrate. For example, in a 100% density treatment, the surface area of the fish equaled the surface area available on the bottom of the tank. Stocking density treatments of 100%, 150% and 200% were used. Fish surface was estimated by tracing live specimens from several different age classes on a 1 cm x 1 cm grid and counting the number of cm^2 grids within each outline. Total length was also measured for each specimen. Since a least square linear regression of length on area gave an $R^2 > 0.9$, it was possible to use total fish length to estimate total fish surface area.

Seven-to nine-month-old summer flounder were stocked into shallow, black plastic 5.5 liter aquaria at densities of 100% (n=8), 150% (n=12), and 200% (n=16). There were three replicates of each treatment. Treatments were connected to a closed-water recirculating system with a water temperature of 18ºC. The flounder were fed Moore-Clark™ formula feed twice daily to satiation. Random samples of fish were measured and weighed every two weeks. Densities were adjusted by increasing tank size when necessary. Data were analyzed using one-way analysis of variance.

Overall, the flounder grew in weight and length over the course of the experiment in all treatments. There were no significant differences in mean lengths and weights ($p > 0.05$) during the 5-week experiment except during the second week. Although significant differences ($p < 0.01$) were seen in length (100%=150%>200%), they were not seen later in the experiment, and likely were due to a small sample size. Towards the end of the experiment, a decrease in size was seen in the 100% and 150% treatments. Since the decrease was seen in both length and weight, this anomaly was probably a result of small sample size. The results of this study suggest that the highest density treatment, 200%, did not have any negative effects on the growth of summer flounder. The optimal stocking density is still unknown.

Cloning and Sequencing of Winter Flounder Digestive Enzyme Genes

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The ontogeny of digestive enzyme function in flatfish has been studied using histochemistry and biochemistry but very little is known at the molecular biological level. Since larval winter flounder are very difficult to wean onto artificial diets at an early age, it is of great interest to know the timing of digesting enzyme expression during development. Molecular techniques such as reverse transcription-PCR (RT-PCR) provide a very sensitive measure of gene expression from minute quantities of tissue or even single cells. In order to acquire the information necessary to establish RT-PCR assays for larval winter flounder, we are isolating cDNA clones for various digestive enzymes.

Three cDNA libraries have been constructed from intestine, pyloric caeca and pancreas in the ëZAP vector (Stratagene). Portions of the trypsin, elastase, aminopeptidase and amylase genes have been prepared by PCR amplification using primers based on conserved sequence motifs, and their identity confirmed by sequencing. The PCR products were cloned into the plasmid vector pCR2.1 (Invitrogen) and used as radioactively-labeled probes to screen the ëZAP libraries. This has resulted in the isolation of several cDNA clones for elastase, trypsin, and aminopeptidase and represent the first cDNAs for winter flounder digestive enzymes, as well as the first elastase and aminopeptidase cDNAs for any fish. Sequence analysis of these clones will be presented as well as Southern hybridization analysis of gene copy number. Screening for amylase cDNAs is in progress.

From this sequence information, primers can now be designed that can be used to specifically amplify coding sequences of digestive enzymes of interest from whole larvae at different developmental states by RT-PCR. Probes will also be developed that can be used for *in situ* electron microscopy to identify which cells or portions of the larval gut are producing specific enzymes. These will be invaluable tools for estimating when larval digestive tracts are competent to assimilate exogenous food, and investigating genetic, physical or environmental parameters affecting digestive function.

Juvenile Winter Flounder Injected Subcutaneously with Elastomer: Movements and Mortality Estimates in Two Selected Coastal Salt Ponds

C. L. Gray and M. R. Gibson

Rhode Island Fish and Wildlife
Marine Fisheries Section
150 Fowler Street
Wickford, RI 02582

Limited movement of young-of-the-year (YOY) winter flounder (*Pleuronectes americanus*) was found from May through October of 1996. During 1996, YOY winter flounder were tagged subcutaneously with visible impact fluorescent elastomer (VIE) and released at one station in Point Judith Pond and the Narrow River, in conjunction with the ongoing juvenile finfish survey being conducted in Rhode Island coastal ponds. Recaptures occurred during all months. These were 906 and 917 YOY winter flounder tagged in Point Judith Pond and the Narrow River, respectively. Of the recaptured fish, 100 percent were within the tagging station. None were found at any of the regular juvenile survey stations in Point Judith Pond or the Narrow River, suggesting limited movement. Survival estimates will be calculated when the data set is completed.

Rearing Juvenile Summer Flounder, *Paralichthys dentatus,* in Different Temperature and Salinity Combinations to Enhance Growth for Aquaculture

G. Klein-MacPhee[1] and M. O'Connor[2]

[1]*University of Rhode Island*
Graduate School of Oceanography
Narragansett, RI 02882-1197

[2]*University of Rhode Island*
Department of Resource Development
College of Fisheries, Aquaculture, and Veterinary Science
Kingston, RI 02880

Juvenile flounder were reared on a commercial diet under different temperature and salinity regimes to determine best growth conditions for aquaculture, and whether the fish could be reared at low salinities. Temperatures used were 16, 20, and 24ºC; salinities 10, 20 and 30 ppt. Significant differences in growth occurred. Two-way ANOVA showed significant effects of temperature with high temperatures providing the best growth. There were no significant differences between salinities, nor was there a significant interaction between temperature and salinity on growth. Fish at 24ºC increased weight by 320% and length by 53%; those at 20ºC increased weight by 175% and length 34% and at 10ºC increased weight by 130% and length 25%. High levels of nitrogen (nitrite, nitrate, ammonia) occurred in a preliminary experiment which produced some interesting results.

The conclusions are that temperature has a strong effect on growth but salinity does not; thus, fish can be reared at 10 ppt with no apparent loss in growth potential.

Ecology of Sheepscot Bay Winter Flounder

R. Langton, S. Sherman, C. Simard, B. Joule, H. Perkins, and S. Chenoweth

Maine Department of Marine Resources
PO Box 8, West Boothbay Harbor, ME 04575

The winter flounder population in Sheepscot Bay has been studied, from 1988 through 1994, using otter trawl, fyke net and beach-seine surveys at various locations throughout the Bay. We have documented, through the examination of otolith daily growth rings, that winter flounder spawn in the region primarily during June but some fish spawned as late as July. The larvae settle out of the water column and occur as juveniles in intertidal fyke net samples as early as June but may occur in low numbers as late as December. Over the four years of sampling, from 1990 through 1994, a consistent pattern of occurrence was observed. A rapid rise in September was followed by a peak in abundance, usually in October. There was an equally precipitous decline in the catch in November dropping to very low numbers in the December samples. The mean size of fish ranged from 4.0 to 8.0 cm at the peak abundance in September and October.

Trawl catches near Sequin Island, at a depth of 19 fathoms, showed a very strong correlation between winter flounder occurrence and temperature. The flounder catch steadily increased from a low in March to a peak in September and October as the temperature ranged from 1.5 ºC in March to 10.0 ºC in September. Juvenile fish dominated the catch all year with the mean size of 17 cm and a range from 7 to 38 cm in total length. The peaks in abundance at both the intertidal station and the Sequin tow occurred during the autumn, suggesting a strongly temperature-driven system with flounder abundance reaching its low during the late winter and early spring when water temperatures are at their nadir.

As the groundfish stocks continue to decline the question of stock enhancement has arisen. In Maine, a legislatively-created Groundfish Hatchery Study Commission was established to investigate the feasibility of enhancement as a management tool, for example. The basic biological question remains, however, can hatchery-reared groundfish be utilized to augment wild stocks of fish? If enhancement can be demonstrated successfully then the question is one of scale and economics. It is our suggestion that the winter flounder population in Sheepscot Bay may be a model population for researching this question. To this end winter flounder larvae were obtained from Dr. Hunt Howell at the University of New Hampshire this spring. These larvae were reared on algae, rotifers, Artemia, and wild plankton. They metamorphosed after 35 days and continued to grow with very low mortality rates until August. Less than optimal rearing facilities resulted in an increased oxygen demand when we were trying to wean the fish to artificial food and we lost our fish. Nevertheless, Dr. Howell has maintained a culture of juveniles and tentative arrangements were made to tag these fish with visual implant tags for preliminary parallel release and recapture experiments in the Sheepscot and Great Salt Bays. Hatchery rearing of winter flounder will be attempted next year at the Department of Marine Resources Laboratory for more extensive field experimentation.

Light Intensity and Salinity Effects on Eggs and Yolk-sac Larvae of the Summer Flounder *Paralichthys dentatus*

W. O. Watanabe[1], M. W. Feeley[2], S. C. Ellis[1], E. P. Ellis[1], and R. A. Cooper[2]

[1]Caribbean Marine Research Center
805 East 46th Place
Vero Beach, FL 32963

[2]The University of Connecticut
Marine Sciences and Technology Center
1084 Shennecossett Road
Groton, CT 06340-6097

The effects of light intensity and salinity on eggs and yolk-sac larvae of summer flounder, *P. dentatus*, were examined under controlled laboratory conditions. Fertilized eggs (early gastrula stage), obtained by induced spawning of captive broodstock, were stocked (53 eggs/L) into fourty-eight 5-L, translucent containers under light intensities of 0 (constant dark), 500, 1000, and 2,000 lux and at salinities of 26, 31 and 36 g/L. Temperature was 19ºC and photoperiod was 12 L:12 D. Light intensity and salinity produced small, but significant ($P < 0.05$) effects on larval notochord lengths at the 97% yolk-sac absorbed stage (114-131 hr post-fertilization =hpf), at first feeding (129.5-135 hpf), and at yolk exhaustion (153.5-159 hpf), which were generally maximal at low light intensity (500 lux) and high salinity (36 g/L) and minimal at high intensity (2,000 lux) and low salinity (26 g/L). Yolk utilization efficiency declined ($P < 0.01$) with increasing light intensity, presumably due to light-induced activity. Assuming that largest larvae have maximum foraging and escape abilities, a low light intensity (500 lux) and a high salinity of 36 g/L were optimal for eggs and yolk-sac larvae, conditions consistent with shelf waters where eggs and early larvae of summer flounder prevail in nature. High survival (85.1%) to yolk exhaustion under all treatment conditions reflects an adaptability for inshore movement during the pelagic larval phase.

Program and Abstracts

Flatfish Biology Conference

December 1-2, 1998
Mystic, Connecticut

Sponsored by

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts

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Flatfish Biology Workshop
December 1-2, 1998, Mystic, Connecticut

by Conference Steering Committee: Anthony Calabrese (Chair)[1], Allan Beck[2], Jay Burnett[3], Donald Danila[4], Arnold Howe[5], Penelope Howell[6], Ambrose Jearld[3], Chris Powell[7], and Anne Studholme[8]

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Sixth in a series of Flatfish Biology Conferences

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts

February 2008

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Milford, CT

Sponsored by

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National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, MA

Flatfish Biology Workshop

December 1-2, 1998, Best Western Sovereign Hotel, Mystic, Connecticut

Oral Presentations

Tuesday, December 1st

7:30 a.m. **Registration/Coffee, Danish and Muffins**

8:15 a.m. Welcome and Introduction
Anthony Calabrese, Chair
National Marine Fisheries Service
Milford Laboratory
Milford, CT

Michael Sissenwine, Director
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, MA

Session I

Penny Howell, Chair
Connecticut Department of Environmental Protection
Fisheries Division, Old Lyme, CT

8:30 a.m. Comparison of Flatfish Species Abundances and Distributions (1970's versus 1990's) in the Thames River (CT) Estuary
D. Tolderlund and D. Orchard
U. S. Coast Guard Academy, Department of Science, New London, CT

8:50 a.m. Growth and Mortality Rates of Young-of-the-Year Winter Flounder in Narragansett Bay: Length-based Model Revisited
A. DeLong[1], J. Collie[1], C. Meise[2], and C. Powell[3]
[1]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI, [2]National Marine Fisheries Service, James J. Howard Marine Science Laboratory, Highlands, NJ, and [3]Rhode Island Department of Environmental Management, Division of Fish and Wildlife, Wickford, RI

9:10 a.m. Evidence of Tidal Period Migration for Winter Flounder, *Pseudopleuronectes americanus*, in a Southern New Jersey Estuary
M. C. Curran[1], R. Chant[2], K. Able[3], and S. Glenn[2]
[1]University of South Carolina, Beaufort, SC, [2]Rutgers University, Institute of Marine and Coastal Studies, New Brunswick, NJ, and [3]Rutgers University, Marine Field Station, Tuckerton, NJ

9:30 a.m. Estimating the Abundance of Winter Flounder Spawning in the Niantic River, CT
D. Danila
Northeast Utilities Environmental Laboratory, Waterford, CT

9:50 a.m. Apparent Compensatory Mechanisms During the Egg and Larval Stages of Winter Flounder
J. D. Miller
Northeast Utilities Environmental Laboratory, Waterford, CT

10:10 a.m. **Coffee/Danish/Muffins**

Session II

Don Danila, Chair
Northeast Utilities Environmental Laboratory
Waterford, CT

10:30 a.m. The Role of Intra- and Inter-individual Variability on Consumption Rates of Recently Metamorphosed Winter Flounder, *Pseudopleuronectes americanus*
M. Walsh, D. Witting, and C. Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

10:50 a.m. Morphological Transitions During the Early Ontogeny of Windowpane, *Scophthalmus aquosus*
M. Neuman and K. Able
Rutgers University, Marine Field Station, Tuckerton, NJ

11:10 a.m. Dermatitis of Juvenile Summer Flounder
R. Smolowitz[1], E. Baker[2], and R. Bullis[1]
[1]Marine Biological Laboratory, Laboratory for Aquatic Animal Medicine and Pathology, Woods Hole, MA and [2]PVC Corporation, Fall River, MA

11:30 a.m. Assessment of Young-of-the-Year Winter Flounder Habitat using RNA as an Index of Condition
C. Kuropat[1], R. Mercaldo-Allen[1], R. Goldberg[1], F. Thurberg[1], and B. Phelan[2]
[1]National Marine Fisheries Service, Milford Laboratory, Milford, CT, and [2]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

11:50 a.m. Glycine Stimulates Winter Flounder Renal Tubule Organic Anion Secretion
L. Renfro[1, 2], D. Miller[1, 3], M. Dawson[2], S. Lechter[1], S. Fujukawa[1], and B. Toomey[1, 3]
[1]Mount Desert Island Biological Laboratory, Salsbury Cove, ME, [2]University of Connecticut, Department of Physiology and Neurobiology, Storrs, CT, and [3]NIH-NIEHS Laboratory of Pharmacology and Chemistry, Research Triangle Park, NC

12:10 p.m. **Hosted Buffet Lunch**

Session III

Ambrose Jearld, Chair
National Marine Fisheries Service
Woods Hole, MA

1:10 p.m. Size-specific Predation on Juvenile Summer Flounder, *Paralichthys dentatus*, and the Duration of the Window of Vulnerability
S. Barbeau[1], C. Chambers[2], D. Witting[2], and K. Able[3]
[1]Rutgers University, Graduate Program in Ecology and Evolution, New Brunswick, NJ, [2]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [3]Rutgers University, Marine Field Station, Tuckerton, NJ

1:30 p.m. A Microbiological Survey of Larval Summer Flounder and their Culture Environment at a Commercial Aquaculture Facility
S. Eddy[1], S. Jones[1], G. Nardi[2], and B. Summer-Brason[1]
[1]University of New Hampshire, Jackson Estuarine Laboratory, Durham, NH, and [2]Great Bay Aquafarms, Portsmouth, NH

1:50 p.m. How do Witch Flounder Cope with an Extended Larval Period?
J. Rabe and J. Brown
Memorial University of Newfoundland, Ocean Sciences Centre, St. John's, Newfoundland, Canada

2:10 p.m. Dietary Variation in Age-0 Winter Flounder in a New Jersey Estuary
L. Stehlik and C. Meise
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

2:30 p.m. Species-specific Predation on Natural Zooplankton by Newly-settled Winter Flounder, *Pseudopleuronectes americanus*
P. Shaheen[1], L. Stehlik[2], and J. Manderson[2]
[1]Rutgers University, Institute of Marine and Coastal Sciences, New Brunswick, NJ, and [2]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

2:50 p.m. **Refreshment Break**

Session IV

Anne Studholme, Chair
National Marine Fisheries Service
Highlands, NJ

3:10 p.m. Field and Laboratory Observations on Spawning, Feeding, and Locomotion in Winter Flounder
A. Stoner, A. Bejda, J. Manderson, B. Phelan, L. Stehlik, and J. Pessutti
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

3:30 p.m. Variation in Reproductive Output of Mid-Atlantic Bight Flatfishes
P. Berrien and C. Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

3:50 p.m. How Important is the Green Crab, *Carcinus maenas*, as a Predator of YOY Winter Flounder, *Pseudopleuronectes americanus*?
E. Fairchild and W. H. Howell
University of New Hampshire, Coastal Marine Laboratory, Durham, NH

4:10 p.m. Substratum Preference by Young-of-the-Year Winter Flounder, *Pseudopleuronectes americanus,* and the Influence of Food
B. Phelan and J. Manderson
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

4:30 p.m. Predation by Striped Searobin, *Prionotis evolans*, on Age-0 Winter Flounder, *Pseudopleuronectes americanus,* and an Alternative Benthic Invertebrate Prey, *Crangon septemspinosa*
J. Manderson[1], B. Phelan[1], L. Stehlik[1], A. Bejda[1], and M. Nunez[2]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [2]Rutgers University, Institute of Coastal and Marine Sciences, New Brunswick, NJ

4:50 p.m. **Poster set-up**

6:00 p.m. **Hosted Mixer and Poster Session**

Wednesday, December 2nd

7:45 a.m. **Registration/Coffee, Danish and Muffins**

Session V

Chris Powell, Chair
Rhode Island Division of Environmental Management
Wickford, RI

8:10 a.m. Using Winter Flounder Growth Rates to Assess Habitat Quality Across an Anthropogenic Gradient in Narragansett Bay, RI
C. Powell[1] and L. Meng[2]
[1]Rhode Island Division of Environmental Management, Fish and Wildlife, Wickford, RI, and [2]U. S. Environmental Protection Agency, Atlantic Ecology Division, Narragansett, RI

8:30 a.m. Comparative Survival and Behavior of Hatchery-reared versus Wild Summer Flounder: A Laboratory Approach
T. Kellison
North Carolina State University, Department of Marine, Earth, and Atmospheric Sciences, Raleigh, NC

8:50 a.m. Habitat Utilization of Juvenile Summer Flounder in the Virginia Portion of the Chesapeake Bay
R. Kraus and J. Musick
Virginia Institute of Marine Science, School of Marine Science, Gloucester Point, VA

9:10 a.m. Comparison of Models for Defining Nearshore Flatfish Nursery Areas of Flatfishes in Alaskan Waters
B. Norcross, A. Blanchard, and B. Holladay
University of Alaska Fairbanks, Institute of Marine Science, Fairbanks, AK

9: 30 a.m. Habitat-specific Utilization of Nursery Grounds in Juvenile Summer Flounder, *Paralichthys dentatus*: A Mark-recapture Experiment
J. C. Taylor
North Carolina State University, Department of Zoology, Raleigh, NC

9:50 a.m. A Long-term Study of Settlement and Growth Patterns in Young-of-the-Year Winter Flounder in New Jersey Estuaries
S. Sogard[1] and K. Able[2]
[1]National Marine Fisheries Service, Hatfield Marine Science Center, Newport, OR, and [2]Rutgers University, Marine Field Station, Tuckerton, NJ

10:10 a.m. **Coffee/Danish/Muffins**

Session VI

Jay Burnett, Chair
National Marine Fisheries Service
Woods Hole, MA

10:30 a.m. Quantifying Ontogenetic Change: A Multivariate Analysis of Larval Development in Flatfishes
H. Hamlin, C. Chambers, and D. Witting
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

10:50 a.m. Effects of Pier Shading on the Growth of Juvenile Winter Flounder, *Pseudopleuronectes americanus*
J. Duffy-Anderson and K. Able
Rutgers University, Marine Field Station, Tuckerton, NJ

11:10 a.m. The Interaction of Turbidity and Substrate Preferenda of Winter Flounder, *Pseudopleuronectes americanus*
P. Grecay and K. Stierhoff
Salisbury State University, Department of Biological Sciences, Salisbury, MD

11:30 a.m. Revisions in Flatfish Taxonomy: Sometimes the Names Have to Change
J. A. Cooper
National Marine Fisheries Service, Systematics Laboratory, Museum of Natural History, Washington, DC

11:50 a.m. Using Coded Wire Tags to Study Movement and Growth of Young-of-the-Year Winter Flounder, *Pseudoleuronectes americanus*, in Point Judith Pond, Rhode Island
R. Young-Morse and C. Recksiek
University of Rhode Island, Department of Fisheries, Animal, and Veterinary Science, Kingston, RI

12:10 p.m. **Hosted Buffet Lunch**

Session VII

Arnold Howe, Chair
Massachusetts Division of Marine Fisheries
Pocasset, MA

1:10 p.m. The Foraging Ecology of Yellowtail Flounder, *Limanda ferruginea*, Larvae: Inferences from Laboratory Studies
J. Brown and V. Puvanendran
Memorial University of Newfoundland, Ocean Sciences Centre, St. John's, Newfoundland, Canada

1:30 p.m. The Effects of Temperature and Salinity on Feeding, Growth, and Survival of Juvenile Summer and Southern Flounder, with a Comparison of Salinity Preference
U. Howson and T. Targett
University of Delaware, Graduate School of Marine Studies, Lewes, DE

1:50 p.m. Benefits of Green Water: Effects on Growth, Survival and Time to Metamorphosis of Winter Flounder, *Pseudopleuronectes americanus*
D. Bidwell and W. H. Howell
University of New Hampshire, Coastal Marine Laboratory, New Castle, NH

2:10 p.m. The Effects of Feeding Schedule and Frequency of Feeding on Summer Flounder Juvenile Growth in Recirculating Systems
G. Klein-MacPhee[1], B. Murphy[2], and E. Rectisky[2]
[1]*University of Rhode Island, Graduate School of Oceanography, Narragansett, RI, and*
[2]*University of Rhode Island, Department of Fisheries, Animal and Veterinary Science, Kingston, RI*

2:30 p.m. Variation in Temperature Effects on Embryonic and Early Larval Period Attributes of Winter Flounder, *Pseudopleuronectes americanus*
C. Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

2:50 p.m. **Refreshment Break**

Session VIII

Allan Beck
Environmental Advantage Group
Prudence Island, RI

3:10 p.m. The Basis and Potential Utility of Meristic and Morphological Variation in Winter Flounder, *Pseudopleuronectes americanus*
D. Witting and C. Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

3:30 p.m. Survival of Larval Winter Flounder, *Pseudopleuronectes americanus:* Evidence for a Critical Period?
S. Lewis, C. Chambers, and D. Witting
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

3:50 p.m. Effect of Dietary Protein Level on the Growth, Survival, and Feed Performance of Juvenile Summer Flounder, *Paralichthys dentatus*
N. King and W. H. Howell
University of New Hampshire, Department of Zoology, Durham, NH

4:10 p.m. Larval Rearing Techniques of Yellowtail Flounder
V. Puvanendran, D. Boyce, N. Morris, and J. Brown
Memorial University of Newfoundland, Ocean Sciences Centre, St. John's, Newfoundland, Canada

Poster Session
Tuesday, December 1st, 6:00 p.m.

Post-metamorphic Growth of Summer Flounder in Laboratory Culture: Do Early-settling Larvae Grow Faster than Late Settlers?
T. Simlick, R. Katersky, N. Marcaccio, and D. Bengtson
University of Rhode Island, Department of Fisheries, Animal and Veterinary Science, Kingston, RI

Effect of Photoperiod on Survival, Growth and Pigmentation of Summer Flounder, *Paralichthys dentatus*, Larvae in Laboratory Culture
M. Huber, E. Moore, N. Maraccio, R. Katersky, and D. Bengston
University of Rhode Island, Department of Fisheries, Animal and Veterinary Science, Kingston, RI

Otogenetic Diet Shifts of Larval and Juvenile Flatfish: Estimating Turnover Rates with Stable-Isotope Ratios
K. Bosley[1], D. Witting[2], C. Chambers[2], and S. Wainright[1]
[1]Rutgers University, Institute of Marine and Coastal Science, New Brunswick, NJ, and [2]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

Molecular Characterization of Ribosomal DNA from Representative Flatfishes of the U.S. Atlantic Coast
P. Kar[1], Z. M. G. Sarwar. Jahangir[2], and R. Eckhardt[1]
[1]Brooklyn College of CUNY, Brooklyn, NY, and [2]The Richard Stockton College of New Jersey, Pomona, NJ

Size-specific Predation on Recently Metamorphosed Winter Flounder, *Pseudopleuronectes americanus*, and the Duration of Their Vulnerability to *Crangon septemspinosa*
D. Witting and C. Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

Temperature Effects on Age, Size and Condition at Hatching in Windowpane, *Scophthalmus aquosus*
M. Cook and C. Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

The Distribution and Size Composition of Five Flatfish Species in Long Island Sound Based on the Connecticut Fisheries Division Bottom-trawl Survey, 1984-1997
K. Gottschall, M. Johnson, and D. Simpson
Connecticut DEP, Fisheries Division, Old Lyme, CT

Aspects of the Life History of Hogchoker, *Trinectes maculatus*, in Delaware Bay Marsh Creeks
R. Bush and K. Able
Rutgers University Marine Field Station, Tuckerton, NJ

Growth of Young-of-the-Year Winter Flounder, *Pseudopleuronectes americanus*, within Eelgrass, *Zostera marina*: Impact of Habitat Edge
P. Bologna and K. Able
Rutgers University Marine Field Station, Tuckerton, NJ

An Evaluation of the Relationship between Otolith Microstructure, Otolith Growth, and Somatic Growth in a Temperate Flatfish, *Scophthalmus aquosus*
M. Newman[1], D. Witting[2], and K. Able[1]
[1]Rutgers University Marine Field Station, New Brunswick, NJ, and *[2]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ*

Development of Spawning and Rearing Techniques for Southern Flounder in South Carolina
M. Denson and T. Smith
Marine Resources Research Institute, South Carolina Department of Natural Resources, Charleston, SC

Comparison of Diets Among Four Co-occurring Juvenile Flatfishes near Kodiak Island, Alaska
B. Holladay
University of Alaska Fairbanks, Institute of Marine Science, School of Fisheries and Ocean Sciences, Fairbanks, AK

Somatic Growth and Otolith Growth in Juvenile Fringed Flounder *Etropus crossotus*
M. Reichert[1], J. Dean[1], R. Feller[1], and J. Grego[2]
[1]University of South Carolina, Belle W Baruch Institute, Columbia, SC, and [2]University of South Carolina, Department of Statistics, Columbia, SC

Utilization of Intertidal and Marina Habitats by Juvenile Winter Flounder, *Pseudopleuronectes americanus*
M. Mroczka[1], J. Carlson[2], T. Randall[3], and P. Pellegrino[4]
[1]Cedar Island Marina Research Laboratory, Clinton, CT, [2]National Marine Fisheries Service, Panama City, FL, [3]University of Mississippi, Department of Biology, University, MS, and [4]Southern Connecticut State University, Department of Biology, New Haven, CT

Winter Flounder Tagging in Western Cape Cod Bay in the Decade of the 1990s: Movements, Fidelity, and Population Size
R. Lawton, B. Kelly, J. Boardman, and V. Malkoski
Massachusetts Division of Marine Fisheries, Pocasset, MA

Effects of 2, 3, 7, 8- Tetrachloroolibenzo-p-Dioxin on Winter Flounder Embryos from NY/NJ Harbor Estuary and Long Island Sound
K. Cooper
Rutgers University, Department of Biochemistry and Microbiology, New Brunswick, NJ

Abstracts
Oral Presentations

Comparison of Flatfish Species Abundances and Distributions (1970s versus 1990s) in the Thames River (CT) Estuary

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Bottom trawl and beach seine samples were taken during 1972-1974 and 1992-1998 on the Thames River (CT) adjacent to the U. S. Coast Guard Academy (river mile 4). The three habitats studied in the estuary included a channel dredged to 41 ft (sta. 60), a 10-ft shoal site (sta. 65), and a sandy beach at Jacob's Rock (JR). Principal flatfish observed include winter flounder (*Pseudopleuronectes americanus*), summer flounder (*Paralichthys dentatus*), windowpane (*Scophthalmus aquosus*), fourspot flounder (*Paralichthys oblongus*), and hogchocker (*Trinectes maculatus*). Our study focused on a comparison of the flatfish population distributions in terms of species and size as related to habitat, season, and year.

Growth and Mortality Rates of Young-of-the-Year Winter Flounder in Narragansett Bay: Length-based Model Revisited

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Much attention has focused on determining recruitment of juvenile finfish into the exploitable adult population. To this end, many experiments have attempted to quantify young-of-the-year (YOY) fish abundance and mortality. Most estimates of mortality come from linearized catch curves fit with least squares regressions, which assume that the population is closed and mortality is constant over the time period of interest. Although numerous factors may influence YOY mortality, it is believed that mortality rates and growth rates may decrease as fish size increases. This results in changes in mortality rates throughout the first year of life as the individuals in the population grow. A length-based model has been constructed which quantifies the changes in growth and mortality rates of YOY winter flounder. We used a modified version of this model to quantify growth and mortality rates of YOY winter flounder using 1988 to 1998 CPUE estimates from the Rhode Island Division of Fish and Wildlife beach seine survey in Narragansett Bay. We attempt to explain year-to-year variability in these estimates by considering both temperature and density-dependent growth and mortality.

Evidence of Tidal Period Migration for Winter Flounder, *Pseudopleuronectes americanus*, in a Southern New Jersey Estuary

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Our prior research has indicated that coves near Little Egg Harbor Inlet, NJ are settlement areas for winter flounder, *Pseudopleuronectes americanus*, and that estuarine circulation patterns in this well-mixed, flood-dominated system support the advection of larvae into these coves. In 1997, we performed both surface and bottom plankton tows synchronously with repeated Acoustic Doppler Current Profiler (ADCP) transects over the study area. Results indicate that a flow separation (tidally driven eddy) near the inlet traps estuarine water during flooding current and that coves tend to be filled with this water. Therefore, larvae hatched in the estuarine spawning ground are transported toward the inlet during the ebb, but during the flood these larvae are trapped by the flow separation and are advected into the cove. However despite tidal currents approaching 2 m/s, our observations indicate that the temporal variability of larval abundances cannot be explained solely by horizontal advection from an estuarine source. In fact, the larval abundances are highest during the flood when salinities indicate waters are of an offshore origin. Three possibilities are discussed to explain this temporal variability; 1) an offshore source of winter flounder larvae, 2) enhanced vertical mixing during the stronger flood tide, or 3) tidal period vertical migration. An offshore source is discounted based on what is currently known about winter flounder spawning. Results from numerical simulations indicate that ebb to flood asymmetries in vertical mixing, though present, cannot explain the enhanced larval abundance during flooding current. Subsequently we conclude that enhanced larval abundances during the flood is evidence of tidally-influenced vertical migration. Larvae that migrate to the bottom of the channel during the ebb may avoid being swept out the inlet and instead may rise to the surface during the subsequent flood to find suitable settlement habitat, in this instance within coves, in the estuary.

Estimating the Abundance of Winter Flounder Spawning in the Niantic River, CT

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The winter flounder is a coastal flatfish most abundant in the central portion of its range, including Long Island Sound. Its population is subdivided into a number of stocks associated with specific estuaries or coastal areas, and adults tend to faithfully return to natal estuaries to spawn each winter. Northeast Utilities has monitored the abundance of adult winter flounder spawning in the Niantic River, CT embayment during mid-February to early April of each year since 1976. Winter flounder are captured using a 9.1-m otter trawl and all fish larger than 20 cm are marked with a brand chilled in liquid nitrogen before returning them to the river. Annually, relative abundance is characterized by trawl catch-per-unit-effort (CPUE) and absolute abundance estimates have been generated since 1984 using the mark and recapture data and the Jolly model for open populations. Information on sex ratio, length-frequency distribution, and spawning condition is also recorded during each survey. Using a length-fecundity relationship, annual egg production estimates have also been calculated.

Although the two abundance estimates are independent of one another, CPUE and absolute abundance estimates are highly correlated. Abundance of Niantic River winter flounder peaked in the early 1980s; but as fishing mortality increased to high levels in the late 1980's, abundance declined thereafter and presently is very low. Despite small numbers of spawners at present, adult winter flounder remain capable of producing large numbers of larvae, which under appropriate conditions can result in a relatively strong year-class of juveniles.

Apparent Compensatory Mechanisms during the Egg and Larval Stages of Winter Flounder

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The Niantic River winter flounder spawning stock has been declining since the mid-1980s based on annual population estimates from mark and recapture studies conducted during their spawning season, primarily February through early April. In addition, larval winter flounder abundance, growth and mortality were monitored from February through early June 1994. As expected, total egg production in the Niantic River has decreased concurrent with the decline of spawning adults. From 1984 through 1994 the annual abundances of yolk-sac larvae were positively correlated with annual total egg production estimates, suggesting that egg survival was similar among these years. However, the abundance of newly hatched larvae from 1995 through 1997 appeared to be greater than expected from this relationship, with egg survival increasing as production declined to relatively low levels. Possibly, egg survival is a compensatory mechanism, where egg abundance less than some threshold resulted in less predation pressure, perhaps because of fewer cues for predators. In addition, annual larval mortality rates were compared to total egg production and seasonal water temperatures using a multiple regression model. The best model indicated that larval mortality decreased as egg production decreased and April water temperatures increased. This suggested that density-dependent larval mortality occurred in the Niantic River that was further moderated by April water temperatures. The effect of temperature on mortality may be due to its positive relationship to rates of larval growth and development.

The role of Intra- and Inter-individual Variability on Consumption Rates of Recently Metamorphosed Winter Flounder, *Pseudopleuronectes americanus*

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The rate of prey consumption is a key component of any analysis of fish bioenergetics. We report results from an experimental investigation of variation in consumption rates of recently metamorphosed winter flounder, *Pseudopleuronectes americanus*. Our objectives were 1) estimate the variation in consumption rates among individual fish, 2) assess the contribution of fish body size to the variation in consumption rate, and 3) evaluate the degree to which the variation in consumption rates among individuals was repeatable. By using brine shrimp (*Artemia*) as prey, we conducted a series of short-term (2-hr) inout-output predation trials in which we exposed single winter flounder of varying sizes (9 to 30 mm total length) to constant densities of instar I *Artemia* nauplii. The magnitude of variation in consumption rates among individuals was substantial (from 0 to > 225 nauplii hr^{-1}). The number of nauplii consumed tended to increase with flounder body size, but the clearest relationship existed for the maximum number of nauplii consumed for a given size of flounder. The latter relationship serves as a better estimate of an upper bound on size-specific consumption rates of juvenile winter flounder. The level of intra-individual repeatability in consumption rates was remarkably high (R^2=0.83), indicating that the variation we observed in consumption rates on any given set of trials was not due to within-individual inconsistencies in appetite or capability. We also ran trials of longer duration (24 hr) on a subset of individuals and repeated trials on some individuals at several-week intervals. These extensions showed that our findings from 2-hr trials are predictive of consumption over a 24-hr period and, more importantly, that the consumption rates exhibited in short-term trials early in juvenile life are predictive of a fish's performance at later dates.

Morphological Transitions During the Early Ontogeny of Windowpane, *Scophthalmus aquosus*

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Morphological transitions occurring during the transformation from the larval to the juvenile stage in a temperate flatfish, windowpane, *Scophthalmus aquosus*, were examined in order to gain further insights about the duration and significance of this dynamic period. We collected young-of-the-year (YOY) windowpane in a southern New Jersey estuarine system during spring and fall spawning events (n 158, 4-54 mm TL). Windowpane were characterized according to flexion stage, eye migration, scale formation, fin ray development, pigmentation patterns, lateral line development, and degree of development and ossification of gill rakers, fins and jaws. We found significant differences among larval (about 4-10 mm TL), early demersal (recently settled; about 11-33 mm TL) and juvenile fish (about 34-54 mm TL) in most of these morphological characters. In addition, we found a high degree of variation (CV) in size at the onset and completion of many characters suggesting that windowpane metamorphosis and settlement may occur over a broader size range than previously thought. We propose that the morphological differences among YOY windowpane, in conjunction with behavioral differences identified in an earlier study, may be used to make predictions about the timing of metamorphosis and settlement relative to size of an individual and may serve as markers for distinguishing between ontogenetic stages.

Dermatitis of Juvenile Summer Flounder

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During the period from February 1998 to April 1998, moribund and dead summer flounder, *Paralichthys dentatus*, were submitted to LAAMP for examination. Flounder were submitted by two different grow-out operations from three different locations in Massachusetts, ranging in size from 8-12 cm in length (juveniles). Various conditions were noted, but the two most immediate problems identified were Trichodinal dermatitis of the eyed head and severe ulcerative bacterial dermatitis of the eyed head. Mortality was associated with both problems. In the first case, mortality was constant, low to moderate (10-20 fish per day), and was controlled with formalin treatment. Unfortunately, treatment needed to be repeated on almost a weekly basis to keep the infestation under control. In the second case, mortality was severe and rapid with loss of almost the entire stock in one facility. In these animals severe focally extensive ulcerative dermatitis and cellulitis was noted. Bacteria cultured from the blood and dermal lesions in these animals included several *Vibrio* spp. and a *Pseudomonas* sp. While causes of these disease problems may have appeared to have different origins, some underlying factors were identified, including: the location of the primary lesions; age of the flounder; temperature/season; and start-up status of the submitting facilities.

Assessment of Young-of-the-Year Winter Flounder Habitats Using RNA as an Index of Condition

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Young-of-the-year winter flounder were field-collected and then redeployed in cages within geographically distinct estuaries: the Hammonassett River in Clinton, Connecticut; the Navesink River in Sandy Hook, New Jersey; and Great Bay/Little Egg Harbor in Tuckerton, New Jersey. Cages were placed in one of several different habitat types including eelgrass and macroalgal sites, adjacent unvegetated areas, and a marsh creek. RNA analysis of white muscle tissue was used to assess the influence of habitat type on growth rate. A strong correlation between somatic growth and RNA concentration suggests that RNA is a good measure of growth in juvenile flounder.

Glycine Stimulates Teleost Renal Tubule Organic Anion (OA) Secretion*

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The renal proximal tubule is an important site of secretion of organic anion. Glycine may prevent hypoxic injury in this tissue by a process involving a glycine-gated anion channel. We examined the relationship of OA secretion and glycine in killifish proximal tubules, masses of winter flounder renal tissue, and monolayers of flounder proximal tubule cells in primary culture (PTCs). Uptake of the OA, fluorescein (FL), was determined in isolated tubules and masses; PTCs mounted in Ussing chambers were used to measure 14C-para-aminohippuric acid (PAH) fluxes. Anoxia (N2, 0.5h) ± 5mM glycine was followed by measurement of FL uptake into cellular (C) or luminal (L) compartments during 30 min reoxygenation yielding: control: (C) 29 ± 2.3, (L) 82 ± 9.1; anoxia: (C) 26 ± 1.9, (L) 37 ± 3.6; anoxia + glycine: (C) 38 ± 3.4, (L) 111 ± 10.5. Glycine stimulated at 2 mM and approached maximum effect at 10 mM. Phorbol ester inhibition of FL uptake was prevented by glycine, which stimulated initial uptake 102 ± 38%. PAH transport by PTCs showed leak flux of PAH was unaffected by 5 mM glycine whereas secretory flux was significantly increased 25%. Glycine was most effective when placed in the peritubular side and had no effect from the luminal side. Strychnine was as effective as glycine in the ìM range, and the effects of glycine and strychnine were not additive. Glycine significantly stimulated organic anion secretion through several possible pathways.

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Size-specific Predation on Juvenile Summer Flounder, *Paralichthys dentatus,* and the Duration of the Window of Vulnerability

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Predation is a major source of mortality during the early life history of many marine fishes. In the juvenile stage, recently metamorphosed flatfish may be especially susceptible to predation. Even then, however, the outcome of an encounter between predator and prey is likely to be determined by their relative body sizes. To investigate size-specific predation risk we: 1) quantified the influences of body sizes on the probability of predation on juvenile summer flounder, *Paralichthys dentatus,* 2) compared the vulnerability of summer flounder to two different predators, sevenspine bay shrimp, *Crangon septemspinosa*, and blue crab, *Callinectes sapidus*, and 3) determined how temperature effects summer flounder growth rates, and, therefore, the duration of time that they are vulnerable to a given size of predator. We reared recently metamorphosed summer flounder at multiple temperatures to provide us with a range of sizes of summer flounder for the predation trials and to estimate temperature-dependent growth rate of juvenile summer flounder. To assess the impact of relative body sizes on prey survival, we used one-on-one trials between predator and prey. Results from over 150 trials show that the risk of predation decreased with increasing prey size and decreasing predator size. This size-specific risk of predation and the results of the temperature-dependent growth study allowed us to estimate the duration of time that summer flounder are vulnerable to these predators.

A Microbiological Survey of Larval Summer Flounder and their Culture Environment at a Commercial Aquaculture Facility

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Commercial production of marine finfish larvae and fingerlings can be either adversely or beneficially affected by the microbiology of the culture environment. Diseases caused by pathogenic and opportunistic bacteria can incur substantial losses, while other microorganisms, known as probiotics, can competitively exclude pathogens and aid in the health and nutrition of the fish. At GreatBay Aquafarms, the first commercial facility to grow summer flounder, a microbiological survey of the fish and its culture environment was initiated to identify pathogens and probiotics as part of an overall strategy to improve the health and survival of this fish. Samples of the water, live feed, and fish were collected from a succession of production runs in 1996 and 1997. The samples were processed, diluted and plated on TSA, VAM, TCBS, and marine agar in order to enumerate and identify total heterotrophs, total vibrios, and *Vibrio anquillarum*. Differences in microbial communities at different fish developmental stages and between the live feeds were observed. Total heterotrpohs and vibrios were detected at high concentrations during elevated mortality events. In separate experiments, larval fish were challenged with a potentially pathogenic *Vibrio* sp. previously isolated from sick larval fish by bioencapsulating it in the brine shrimp used as a live feed. This technique resulted in a significant mortality of 40%. The results provide an initial database for determining the role of bacteria in the health of larval summer flounder, as well as a technique for introducing probiotic bacteria into the feed and the fish.

How Do Witch Flounder Cope With an Extended Larval Period?

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The witch flounder (*Glyptocephalus cynoglossus*; grey sole) is a commercially important flatfish in the North Atlantic. It has several interesting life history characteristics including a long larval period and a large size at metamorphosis. Due to its high market value we are currently investiagting the potential of this species for cold-water aquaculture. Our research focuses on the live prey requirements of witch flounder larvae. Two complementary experiments are underway to determine the prey concentration required for rearing witch flounder larvae. These experiments can also help elucidate how this species copes with an extended larval period in the wild.

In experiment 1, witch flounder larvae were reared at three different live prey densities (2000, 4000, and 8000 prey/liter) in 33 L flow-through glass aquaria. The density of live prey (enriched rotifers and *Artemia*) was adjusted to the nominal density three times daily. Larvae were sampled weekly for growth and mortality was recorded daily.

Experiment 2 focused primarily on the foraging behavior of witch flounder larvae in response to different prey densities. Twice weekly, larvae were removed from a general rearing tank and placed in 2-L glass bowls containing different concentrations of prey (250, 500, 1000, 2000, 4000, 8000, and 16000 prey/L). After a 5-minute acclimation period the foraging behavior of individual larvae was recorded for 2 minutes.

Preliminary results supports the idea that the prey requirements of witch flounder larvae are lower compared to other cold-water flatfish, which may relate to higher assimilation efficiencies or lower requirements for growth.

Dietary Variation of Age-0 Winter Flounder in a New Jersey Estuary

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Potential sources of dietary variation in fishes include ontogeny (size), season, and location. To explore dietary variation in young-of-the-year winter flounder, we collected fish by beam and otter trawl during a 2-year, 84-station survey of the Navesink River-Sandy Hook Bay estuary. Diet items were analyzed using cluster analysis by predator size class and season. Cluster analysis indicated that the young fish could be divided into two size groups: 10-60 mm and 61-120 mm.

Diets varied both spatially and temporarily within size class. For example, in May 1997, calanoid copepods dominated the diet of very small (10-60 mm) fish in the river; while spionid and other polychaetes were the primary prey items at most stations in the bay. Concurrently, diets of larger fish in both river and bay samples contained a wider variety of organisms, including large portions of spionids and ampeliscid amphipods. Diets in July 1997 were similar, with the addition of gammarid amphipods and various polychaetes, and the omission of calanoids. At a few bay stations, diets were composed mainly of ampeliscids. Dietary variation is examined in the context of spatial and temporal factors and the availability of prey as determined from benthic samples.

Species-specific Predation on Natural Zooplankton by Newly-settled Winter Flounder, *Pseudopleuronectes americanus*

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The consumption of natural zooplankton by newly-settled winter flounder, *Pseudopleuronectes americanus*, was investigated in the Navesink River-Sandy Hook Bay estuarine system, NJ, in May-June, 1998. The study was initiated because an examination of the stomach contents from 155 newly-settled winter flounder collected in a May 1997 survey had yielded puzzling results: (1) the conspicuous absence of the calanoid copepod, *Acartia tonsa*, an historically abundant late spring species; and (2) the presence of the calanoid copepod, *Eurytemora affinis*, which usually is not numerically dominant in late spring. The epibenthic zooplankton community was assessed by sampling nine transects, with 27 stations, using a closing plankton net. Newly-settled winter flounder were collected concurrently with otter and beam trawls. Zooplankton analysis showed *A. tonsa* and *E. affinis* as the major components of the holoplankton throughout the system. Although the abundance of *A. tonsa* was substantial from the head to the middle of the river, *E. affinis* was numerically dominant. *A. tonsa* was the most abundant in the bay, with few *E. affinis* observed. *A. tonsa* was never consumed by newly-settled winter flounder even while abundant in the plankton, corroborating findings from the 1997 survey. *E. affinis* was the sole calanoid copepod found in the newly-settled winter flounder stomachs during the 1998 study. The feeding behavior persisted even where *E. affinis* was low in numbers in the plankton, suggesting a species- specific predation. *A. tonsa* is the most abundant calanoid copepod in northeastern United States estuaries from late spring to early winter, with a year-long numerical dominance which surpasses that of E. *affinis*. Our findings suggest that newly-settled winter flounder do not utilize or rely upon one of the most abundant food resources in North American estuarine systems.

Field and Laboratory Observations on Spawning Feeding and Locomotion in Winter Flounder

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Field collections in the Navesink River estuary (New Jersey) and long-term observations in a large research aquarium (121 kl) were combined to explore the behavior of winter flounder before, during, and after the spawning season. Males and females arrived in the estuary in ripe spawning condition, and fish with high gonadosomatic indices were collected during most of February and March, 1997. All of the ripe females were >20 cm in total length and ≥ 2 years old, whereas ripe males were collected as small as 10 cm (age 1). In the field, females began feeding earlier in the season than males, primarily on siphons of the clam *Mya arenaria* and on ampeliscid amphipods. In the laboratory, males began feeding only after spawning had ended.

Ten male and ten female winter flounder were videotaped continuously in the research aquarium. During the pre-spawning period, the fish were inactive at 20 ºC, except for short feeding bouts. Thereafter, locomotor activity, swimming speed, and feeding frequency were all inversely related to declining water temperature. Males and females were strictly nocturnal during the reproductive season, but became active during the day and night during the post-spawning season. The flounder spawned over a 60-day period at 4 ºC, with an average of 40 spawns per female and 147 spawns per male. Spawning, always initiated by males, occurred primarily between sunset and midnight. Paired spawning was not common (22% of events) because spawning behavior frequently elicited rapid approaches and group spawning by as many as six secondary males. Multiple parentage would appear to be the outcome of most spawning events, and it is clear that male spawning strategy is adapted for maximum fertilization of eggs.

Variation in Reproductive Output of Mid-Atlantic Bight Flatfishes

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Reproduction is critical to the series of events that eventually determines the level of recruitment in fish populations. Although both reproductive output and recruitment vary, whether they co-vary in any repeatable way remains a largely unresolved question. A fundamental step in answering this question is to ensure that the variation in reproductive output has been accurately depicted. In this project, we use historic databases and archived ichthyoplankton collections to assess the patterns and sources of variation in reproductive output. We consider 'reproductive output' to include measures such as 1) the duration of the spawning season, 2) the amount of eggs produced during the spawning season, 3) the within-season pattern of egg production, and 4) the quality of eggs as can be deduced from their sizes. We have used information from MARMAP and other data bases, and original size measurements from archived icthyoplankton samples, to initiate these retroactive analyses. The first set of species that we have considered are flatfishes that are abundant in shelf waters of the NW Atlantic. Here we present preliminary results on variation in reproductive output of summer flounder, *Paralichthys dentatus*, yellowtail flounder, *Limanda ferruginea*, and windowpane, *Scophthalmus aquosus*, giving emphasis to alternative hypotheses concerning changes in egg sizes during the course of the spawning season. We conclude that seasonal trends in egg size are likely to be driven by seasonal changes in water temperature.

How Important is the Green Crab, *Carcinus maenas*, as a Predator of YOY Winter Flounder, *Pseudopleuronectes americanus*?

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As young-of-the-year (YOY) flatfish grow, they achieve size refuges in which they are no longer vulnerable to a series of predators. It has been shown, for example, that from settlement to 20 mm TL, YOY winter flounder, *Pseudopleuronectes americanus*, are vulnerable to predation by the sevenspine bay shrimp, *Crangon septemspinosa*. Once beyond this period of shrimp predation, it has been suggested that the fish enter into another period in which they are susceptible to predation by green crabs, *Carcinus maenas.* To investigate this, a 5 x 6 factorial predator-prey size relationship experiment was conducted at the University of New Hampshire's Coastal Marine Laboratory. Five winter flounder size- class treatments (11-20, 21-30, 31-40, 41-50, and 51-60 mm TL) were tested against five green crab size-class treatments (11-20, 21-30, 31-40, 41-50, and 51-60 mm carapace width). Five replicate trials were conducted for each combination, and a control treatment of flounder only (no crabs) ensured all fish mortality was due to predation. Preliminary data show that green crabs greater than 20 mm prey on YOY winter flounder. Flounder of all size classes were preyed on by green crabs, however, as expected, mortality was highest in large crab-small fish combinations.

Substratum Preference by Young-of-the Year Winter Flounder, *Pseudopleuronectes americanus*, and the Influence of Food

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Laboratory and field results were used to determine the role of sediment selection in habitat choice. Young-of-the-year winter flounder, ranging in size from 15 to 69 mm, were separated into 10-mm size groups. Winter flounder were then given a choice of sediment types over a 24-hr period in circular tanks with filtered flow-through seawater. Small individuals (< 40 mm SL) preferred finer-grained sediments, while larger individuals (≥40 mm SL) preferred coarser-grained sediments. No size groups preferred sediments that prevented burial. These results were then compared to field data on the abundance and distribution of juvenile winter flounder synoptically collected with sediments samples from the Navesink River/Sandy Hook Bay estuarine system in New Jersey. In the field, variation in abundance and distribution was related to sediment characteristics (grain size and percent organics) during the 1997 settlement period. However, these patterns changed temporally along with an increase in winter flounder size. Other laboratory experiments determined that the presence of a live food source, the softshell *Mya arenaria*, was sufficient to influence young-of-the-year winter flounder sediment selection, indicating that food forms another significant part of the distributional relationship.

Predation by Striped Searobin (*Prionotus evolans*) on Age-0 Winter Flounder (*Pseudopleuronectes americanus*) and an Alternative Invertebrate Prey (*Crangon septemspinosa*)

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Laboratory experiments and data from gillnet and otter trawl surveys in the Navesink River/Sandy Hook Bay estuarine system, New Jersey, were used to examine the importance of striped searobin, *Prionotus evolans,* as predators of age-0 winter flounder, *Pseudopleuronectes americanus.* Although crustaceans (primarily sand shrimp*, Crangon septemspinosa)* were the primary food item in searobin stomachs, large numbers of age-0 winter flounder occurred in diets of fish collected in shallow (<5 m) nearshore areas in Sandy Hook Bay. Winter flounder (12-48 mm standard length [SL]), occurred in 68% of searobins collected in a single gillnet sample (N=35, 152-315 mm SL), with individual fish consuming as many as 11 winter flounders. Laboratory experiments conducted to determine body size relationships between striped searobin predators (185-270 mm SL) and winter flounder prey (20-108 mm SL) showed that the maximum size of prey consumed generally increased with predator size and 95% of prey/predator body size ratios were below 0.28 (Range 0.10-0.40). During day and night prey selection experiments, in which equal numbers of winter flounder (30-54 mm SL) and sand shrimp (30-50 mm TL) were offered to searobins, prey consumption was higher during daytime experiments, but prey choice was random. Searobin prey choice was also random in daytime switching experiments (flounder:shrimp; 5:15, 10:10 and 15:5). Video observations showed that searobins used modified pectoral fins to locate and flush winter flounder during most successful attacks. These results suggest that striped searobins are efficient and opportunistic predators of demersal prey and may consume large numbers of age-0 winter flounder when the species co-occur in shallow nearshore areas.

Using Winter Flounder Growth Rates to Assess Habitat Quality across an Anthropogenic Gradient in Narragansett Bay, Rhode Island

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We used winter flounder growth rates to assess habitat quality across an anthropogenic gradient in Narragansett Bay, Rhode Island. Cages (1 m^2) were placed in the Providence River (upper bay), Prudence Island, an estuarine reserve (mid-bay), and Sheffield Cove in the lower bay. Individually marked fish were placed in the cages and growth rates were measured over three approximately two-week experiments from 8 June-22 July. Water temperature, salinity, dissolved oxygen, organic carbon, dissolved inorganic nitrogen, chlorophyll A, and benthic food were also measured. Growth rates ranged from 0.22 to 0.63 mm/day and were generally highest at Prudence Island (mid-bay) and lowest in Sheffield Cove (lower bay). Growth rates were initially highest in the Providence River (the most human-impacted site), but dropped off for the second and third experiments, presumably due to low dissolved oxygen. Growth rates obtained in our Narragansett Bay experiment are similar to those obtained in Rhode Island's coastal ponds, Mount Hope Bay, and in other Northeastern estuaries. Results of this study and previous work suggest winter flounder growth rates are driven primarily by larger forces, such as initial fish size, time of year, and latitude, rather than by habitat type.

Comparative Survival and Behavior of Hatchery-reared Versus Wild Summer Flounder: A Laboratory Approach

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Stock enhancement is receiving increasing attention as a management option to assist in the rebuilding of depleted fishery stocks. Because behavioral patterns of hatchery-reared (HR) fish are often anomalous to those of their wild conspecifics, it is critical to investigate the ability of HR fish to survive in the natural environment before stock enhancement efforts proceed. In the southeastern United States, the summer flounder, *Paralichthys dentatus*, has been identified as an excellent candidate for stock enhancement programs. I describe the use of laboratory trials to compare behavior and susceptibility to predation of HR versus wild juvenile summer flounder.

Predation trials consisted of introducing a blue crab, *Callinectes sapidsus*, as a predator to a tank containing a (1) wild fish, (2) naïve HR fish, or (3) predator-conditioned HR fish. Predation trials were replicated on both sand (n=33/treatment) and mud (n=20/treatment) substrates in an attempt to investigate habitat-specific differences in survival. Trials lasted for 24 h, after which the predator was removed and the fish classified as eaten or not eaten. HR fish suffered significantly higher predation than wild fish, irrespective of substrate type. Predator-conditioned fish exhibited survival rates which were greater than naïve HR fish but less than wild fish. These results were consistent across substrates. In an attempt to understand the mechanisms underlying these survival patterns, replicated behavioral observations were made for both HR and wild fish on sand (n=38) and mud (n=38) habitats. Time spent performing certain behaviors (*e.g.*, swimming in the water column, moving on bottom, buried, *etc.*) was recorded and compared between treatments. HR fish spent significantly more time swimming in the water column than wild fish, whereas wild fish spent significantly more time buried or motionless on the benthos than HR fish. These results suggest that the anomalous behavior patterns of HR fish cause increased susceptibility to predation. Results from the predator-conditioning trials suggest that it may be possible to mitigate behavioral deficits by exposing naïve HR fish to natural stimuli before they are released into natural environments.

Habitat Utilization of Juvenile Summer Flounder in the Virginia Portion of the Chesapeake Bay

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The recent mandate of Essential Fish Habitat (EFH) identification in the Magnuson-Stevens Fishery Management Act, 1997, and the adoption of summer flounder, *Paralichthys dentatus*, as the model species for an EFH identification prototype has required the synthesis and evaluation of available habitat utilization data. Summer flounder is a highly migratory species with a dynamic life history; therefore, ontogenetic stages are treated separately when considering habitat utilization. The highest quality habitat data are available for the inshore juvenile stage, thus most of the work on juvenile flounder habitat has focused on the inshore juvenile habitat. Independent analysis of habitat variables has allowed little comparison of relative effects, although some workers have simultaneously analyzed two or three variables. The objective of this study was to analyze the relative effects of ten variables {temperature, salinity, dissolved oxygen, depth, substrate, slope of the bottom, distance to submerged aquatic vegetation (SAV), distance to the Bay mouth, tide, year}on the occurrence of juvenile summer flounder in the Virginia portion of the Chesapeake Bay through analysis of VIMS long-term unpublished monitoring data. Variables, not measured directly, were applied to the flounder data from various source data using the Geographic Information System (GIS), ARC/INFO. Reduced multiple logistic regression models of the occurrence of summer flounder in catches were developed for each season. Response surfaces were visualized geographically with ARC/INFO by combining generalized source data maps using parameter estimates from the regression models.

Comparison of Models for Defining Nearshore Flatfish Nursery Areas of Flatfishes in Alaskan Waters

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Assessment of nursery habitats of Northeast Pacific flatfishes in Alaska is difficult because of the many thousands of kilometers of coastline. As it is unrealistic to assess fishes in all locations, models are needed to characterize the nursery habitats of flatfish species. Descriptive habitat models of species presence and categorical analysis regression tree (CART) models of species abundance have been developed in previous studies. Based on collections around Kodiak Island, Alaska in 1991 and 1992, these models have been developed for: age-0 flathead sole, *Hippoglossoides elassodon*, age-0 Pacific halibut, *Hippoglossuss stenolepis*, age-1 yellowfin sole, *Pleuronectes asper*, and age-0 rock sole, *Pleuronectes bilineatus*.

In 1995, collections were made in bays along the Alaska Peninsula (an area never before sampled for juvenile flatfishes) separated by 50 km from Kodiak Island across Shelikof Strait, and these collections were compared with the previous models developed for Kodiak Island in 1991 and 1992. Very simple descriptive models (Norcross *et al.* 1995) accurately predicted the presence of flathead sole (78%), Pacific halibut (96%), yellowfin sole (75%), and rock sole (99%) in specific depth ranges and on specific substrate types. More complex CART models of species abundance (Norcross *et al.* 1997) were more precise but not as accurate as the descriptive models because some parameters were not always available at the test locations. Flathead sole were found at temperatures ≤ 8.9 ºC on mud and mixed mud substrates in similar proportions in 1995 (66%) as in 1991-1992 (71%). Similarly, Pacific halibut were ≤ 7.9 km inside bays and at depths ≤ 40 m in 93% of the sites of this study, compared with 89% previously. Seventy-five percent of yellowfin sole were at depths ≤ 28 m on mixed substrates in both study periods. Rock sole were found on sand and muddy sand substrates at temperatures above 8.7 ºC in 52% of the sites in present and 69% of the sites in previous studies.

This field test demonstrated that both descriptive and CART models were very useful at identifying juvenile habitat parameters. However, those models did not fully accomplish the goal of this research, which was the production of simple but reasonably accurate and precise habitat models that can be applied to areas not previously sampled. The resource selection models developed here verified the importance of a subset of parameters used in earlier models and provided a statistical means (78-87% correct) for prediction of fish distribution in similar areas of the eastern North Pacific.

Habitat-specific Utilization of Nursery Grounds in Juvenile Summer Flounder, *Paralichthys dentatus*: A Mark-recapture Experiment

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Juvenile estuarine-dependent fish such as the summer flounder, *Paralichthys dentatus,* utilize a variety of habitats during their early life history. A mark-recapture experiment was used to compare growth, survival and residency in two types of inter-tidal habitats: a high energy sand flat, and a low energy flat adjacent to a Spartina marsh. Fish were tagged and released at the beach (n=117) and marsh site (n=92) from May 12-July 17, 1998. Recapture rates were higher on the beach with 38.5% one-time recaptures and 10 % two-time recaptures. In contrast, the marsh recapture rates were lower, with 17.4% recaptured once and 6.6% recaptured twice. Average residence times were 11 d (range 1-37 d) on the beach and 10 d (range 2-34 d) on the marsh, suggesting that the flounder utilize the beach for longer periods of time than the marsh. Direct measures of growth through tag returns were 0.17 mm/d in the marsh and 0.43 mm/d on the beach. High recapture rates suggest that nonvegetated, inter-tidal flats are critical habitat for juvenile summer flounder; however, apparent differences in growth and residence time between different types of habitat suggest that productivity on flats vary. We plan to expand our research concentrating on factors governing productivity in inter-tidal habitats.

A Long-term Study of Settlement and Growth Patterns in Young-of-the-Year Winter Flounder in New Jersey Estuaries

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The timing of settlement and subsequent growth patterns during the early juvenile stage can potentially regulate survival and year-class strength in marine fishes. We examined spatial and interannual differences in settlement and growth of winter flounder across a nine-year time series. Young-of-the-year fish were collected in late May/early June from four sites spanning the coastline of New Jersey (Sandy Hook, Barnegat Bay, Great Bay, and Wildwood). Overall, size frequency distributions suggested a narrow time period of settlement in late spring. Within this period there were some consistent inter-site and interannual differences in size distributions. For example, when sizes were standardized to a common sampling date of May 30, age-0 fish were smaller in 1994 and 1996 across all sites, suggesting later settlement. Across sites, winter flounder were consistently larger at the southernmost sampling location (Wildwood) and were generally smaller on average at the northernmost site (Sandy Hook). This pattern suggests a seasonal progression of settlement from southern to northern sites, but the trend needs to be confirmed with otolith analysis of settlement dates. Density estimates based on standardized seine tows did not indicate consistent interannnual trends among sites. Sandy Hook typically had the highest densities and Great Bay the lowest densities.

Quantifying Otogenetic Change: A Multivariate Analysis of Larval Development in Flatfishes

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Change in size, shape and features are characteristic of the larval stage of marine teleosts. Ontogenetic change in larval flatfishes is particularly dramatic, as exemplified by the eye migration that is the hallmark of this order. To be accurate, however, it must be recognized that ontogenetic change in larval flatfishes occurs continuously in a multitude of features each with its own rate of transition. The challenge to fisheries ecologists is to portray these changes fairly while generating a larval staging scheme that is of utility for quantifying and interpreting processes in natural populations. Here we review features of various staging schema and provide a framework for reconsidering these in light of flatfish ontogeny and the application of this information to natural populations. We present preliminary data on ontogenetic change in winter flounder, *Pseudopleuronectes americanus*, and windowpane, *Scophthalmus aquosus*, and demonstrate how ontogenetic progressions of multiple features might be best quantified and compared.

Effects of Pier Shading on the Growth of Juvenile Winter Flounder, *Pseudopleuronectes americanus*

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Urban estuaries support human activities and are important areas for fishes. We previously evaluated these potentially conflicting uses by examining growth of juvenile fishes relative to municipal piers in the Hudson River. We found that juvenile winter flounder caged under piers exhibited negative growth while fish in adjacent open water areas grew well. Underpier areas are light-deprived, which may interfere with the feeding ability of visually-feeding fishes. We report the results of experiments designed to examine the effects of shading on feeding and growth of juvenile winter flounder. Two 10-d caging experiments were conducted near a municipal pier in the Hudson River using artificially darkened cages that mimicked the light-deprived conditions of underpier environments. Results suggest that winter flounder may exhibit negative growth in darkened cages, although the effects may be negated when prey availability is not limiting. Feeding success among fishes was evaluated using recently-settled winter flounder in caging experiments of shorter duration. Cages were deployed along an underpier, pier edge, and open water transect for 3 hr, allowing fish an opportunity to feed on available prey items. The contents of the gut were counted and identified, and results indicate both the abundance and diversity of prey consumed may be depressed under piers.

The Interaction of Turbidity and Substrate Preferenda of Winter Flounder, *Pseudopleuronectes americanus*

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Habitat choice by juvenile fish may reflect adaptations which maximize survival. Because young fish are vulnerable to predation, refuge may be an important component of habitat choice. For flatfishes such as the winter flounder, substrates which provide camouflage or enable partial or complete burial are likely to be preferred. Turbidity, a feature of the water column which is ubiquitous in near-bottom environments, may also provide protection from visual predators. However, it is also likely that high turbidity may negatively impact feeding by juvenile winter flounder. The ecological role of turbidity in the habitat choice by winter flounder is unknown. It is possible that in clear conditions juvenile flounder are more vulnerable to predation by visual predators and are thus more likely to prefer substrates which facilitate complete burial. Thus, prevailing conditions of turbidity may affect substrate choice. In this investigation we examined the substrate preferences of juvenile winter flounder in non-turbid conditions. Because turbidity may confer a survival advantage, turbidity preferences and the influence of turbidity on substrate choice were also examined. Turbidities ranged from 0.95 to 11 NTU and represented conditions commonly encountered in estuarine nursery areas.

Revisions in Flatfish Taxonomy: Sometimes the Names Have to Change

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The genus-level taxonomy in the family Pleuronectidae (right-eyed flounders) has undergone significant revisions in recent years. Changes incorporated in the most recent AFS list of common and scientific names (Robins, *et al.* 1991) were based on a phenetic analysis of species interrelationships (Sakamoto 1984). The revisions proposed by Sakamoto's study affected 26 species in 12 genera. Due to the commercial significance of many of these species, the nomenclature (*sensu* Sakamoto) has been the subject of much scrutiny and confusion. Taxonomic arrangements and nomenclatorial changes proposed in this phenetic study have been disputed and revised in the light of new and more complete information based on a cladistic analysis for this family (Cooper and Chapleau 1998). To the nonsystematist, the basis for these changes is sometimes obscure and not well understood. However, nomenclatorial changes mandated by a cladistic analysis, can be meaningful to both taxonomist and fisheries scientists. Direct comparison of these two recent revisions highlights the utility of the cladistic analysis, especially in studies examining comparative biology, ecology and life history of these commercially important flatfish species.

Using Coded Wire Tags to Study Movement and Growth of Young-of-the-Year Winter Flounder, *Pseudopleuronectes americanus*, in Point Judith Pond, Rhode Island

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A project was conducted during a five-month period from July to December 1996 to investigate the use of coded wire tags as a method for studying individual growth and movement of young-of-the-year winter flounder, *Pseudopleuronectes americanus*, in a coastal pond estuary.

Investigations in the lab examined the retention, survival, and effects on growth of this tagging method. Two hundred fish were captured from Narragansett Bay in July and August of 1996, and were kept in flow-through seawater tanks and fed an artificial diet. Retention of the tags was 100% during the four-month study. Survival seemed dependent on size and health of the fish at capture. Of fish measuring greater than 50 mm at capture, survival was 90-100% in all treatment groups. Fish, smaller than 42 mm at capture, had a 42-90% survival rate in all treatment groups, including the control. Growth did not appear to be impacted by tagging, as control groups had similar growth rates to the experimental treatments.

Field trials utilizing this method occurred in two sites of Point Judith Pond, Rhode Island. Fish were captured using seine nets and SCUBA in a 100-m area from August to November, 1996. Fish were subcutaneously injected on the blind side with a coded wire tag, were marked with a panjet ink tattoo to identify fish carrying the tag, and were then released. Recapture attempts occurred one to three weeks after tagging, and were done at the site of release, 50 m down shore, and 100 m down shore. Of the 250 fish marked and injected with coded wire tags, a 5% recovery was observed in the area up to 100 m from the release site. The longest 'at large' recaptured fish was 10 weeks, and upon recapture both the tag and the ink tattoo were still visible, and the fish appeared in good health. Growth measurements appeared consistent with the lab findings, and did not seem to be affected by the tags.

Results indicate that microwire tagging is an effective and efficient means of tagging juvenile fish that are too small for larger external tags where individual data are desired.

The Foraging Ecology of Yellowtail Flounder, *Limanda ferruginea*, Larvae: Inferences from Laboratory Studies

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During the early life stages in fishes, mortality due to starvation is intense. Larval fish are poorly equipped to handle this pressure as they are weak swimmers, have poorly developed sensory and digestive systems, and their behavioral repertoire is limited. Over the past number of years we have been trying to understand how marine fish have adapted to avoid starvation. Our experiments have focused on a number of factors important to larval foraging in the marine environment: light level, prey availability, and type. Our working hypothesis is that marine larvae in the Northwest Atlantic have adapted to "threshold" foraging environments in which their survival is maximized. We have conducted a number of laboratory experiments on yellowtail flounder, *Limanda ferruginea,* larvae to try and define the environments to which they are adapted.

Experimental results suggest that yellowtail larvae require high densities of prey (>4000/l), high light intensity (>2,000 lux) and prefer *Artemia* over rotifers when they reach a length of 7.5 mm. Compared to other marine larvae from the Northwest Atlantic, yellowtail flounder larvae appear to have a similar foraging ecology to Atlantic cod, *Gadus morhua*, but differ significantly from other species.

The Effects of Temperature and Salinity on Feeding, Growth and Survival of Juvenile Summer and Southern Flounder, with a Comparison of Salinity Preference

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Summer flounder, *Paralichthys dentatus*, and southern flounder, *P. lethostigma*, enter SAB estuaries as larvae throughout late winter and early spring. Ontogenetic segregation occurs shortly thereafter, with juvenile southern flounder moving upstream to lower salinity nursery areas and juvenile summer flounder tended to remain at higher salinity areas downstream. To determine the ecological significance of this segregation, as well as to delineate important nursery ground conditions in terms of "essential fish habitat", we examined feeding rate, growth rate, and survival of these two species under different temperature/salinity regimes. Experiments were conducted concurrently on both species at 15, 20, 25 and 30ºC; with *P. dentatus* at 10, 20 and 30 ppt salinity and *P. lethostigma* at 0, 10, 20, and 30 ppt salinity. From 15 to 25ºC growth rates for *P. lethostigma* increased with increasing temperature and decreasing salinity. *P. dentatus* did not survive at 0 ppt. Growth rates for *P. dentatus* were generally unaffected by salinity (10-30 ppt) at 15 and 20ºC, but exhibited a trend of increasing growth with salinity at 25 ºC.

To determine whether salinities that promoted the greatest growth were also preferred behaviorally by each species, salinity preferences between *P. dentatus* and *P. lethostigma* were compared. Circular Staaland devices, modified with ramps for flatfish, were designed to maintain salinity gradients for more than 24 hours. Fish were observed remotely for several hours; each trial was videotaped and percent of time spent at each salinity was determined.

Benefits of Green Water: Effects on Growth, Survival, and Time to Metamorphosis of Winter Flounder, *Pseudopleuronectes americanus*

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The addition of microalgae or "green water" to larval culture systems has recently become an integral part of standard rearing protocol. We examined the effects of varied duration of green water exposure on growth, survival, and time to metamorphosis of larval winter flounder, *Pseudopleuronectes americanus*. Five treatments were employed: clear water (no algae added) and green water addition for 7, 14, 21 and 28 days post-hatch. Algal species utilized were *Duneliella tertiolecta, Nannochloropsis oculata and Tetraselmis suecica.* Larvae from the clear water and the 7-day green water treatments were smaller and grew at slower rates than those in the prolonged green water treatments. At the termination of the experiment larvae exposed to the 21- and 28-day green water treatments had developed further towards metamorphosis than the clear water and the 7-day green water treatments. There were no significant differences in survival between treatments. Based on these results we suggest that green water addition be utilized for the culture of winter flounder larvae at least until the onset of metamorphosis.

The Effects of Feeding Schedule and Frequency of Feeding on Summer Flounder Juvenile Growth in Recirculating Systems

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Two sets of experiments were conducted on laboratory-reared, 5-month-old summer flounder juveniles maintained in recirculating systems. The first set of experiments examined the effects of feeding once or twice a day at different time periods. Since summer flounder have been shown to be night active in both the laboratory and the field, and metabolic rates peaked at night, we hypothesized that feeding more frequently would promote faster growth and that optimum growth rates would occur when fish were fed more frequently at night. One set of fish were fed once a day at 9 AM and 11 PM; the second set of fish were fed twice a day at 9 AM and 3 PM, and at 5 PM and 11 PM. All fish received the same percent body weight of food and there were three replicates for each condition.

Results showed that fish fed twice a day grew significantly faster than fish fed once a day, but there were no significant differences between day and night feedings, and there were no interactions between the two conditions. In the second set of experiments fish were fed once, twice, and four times per day. Fish fed twice and four times per day grew significantly faster than those fed once per day, but there was no significant difference between fish fed twice and those fed four times per day.

Variation in Temperature Effects on Embryonic and Early Larval Period Attributes of Winter Flounder, *Pseudopleuronectes americanus*

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Water temperature can have profound effects on events in the early life history of fishes, including accelerations or delays in development and influences on sizes and energy reserves of larvae. This talk presents analyses of temperature effects on the timing of, and sizes at events in the embryonic and early larval stages of winter flounder, *Pseudopleuronectes americanus,* from multiple locations in the specie's range. By using fertilized eggs of winter flounder from New Jersey, USA, to Newfoundland, Canada, and a standard temperature control apparatus, replicated groups of eggs were incubated at 10 different temperatures (range 0-14ºC). Survival to hatching was high for all but the extreme temperatures. The duration of the embryonic period varied from 4 to more than 40 d, and was log-linearly related to incubation temperature (developmental rate exhibited a slightly convex relationship with temperature). Size at hatching was maximal at intermediate temperatures and was inversely related to the amount of yolk sac reserves at hatching. Duration of the yolk-sac period of larvae held at a common temperature after hatching was influenced by the temperatures that these individuals had experienced during the embryonic period. These patterns of early life history traits and their responses to incubation temperatures are interpreted in the context of 1) events later in larval life, 2) general geographic trends in life history traits, and 3) potential consequences in natural populations.

The Basis and Potential Utility of Meristic and Morphological Variation in Winter Flounder, *Pseudopleuronectes americanus*

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Meristic and morphological traits have long been used to differentiate species and stocks within species. One shortcoming of such analyses is that these traits can be influenced by environmental conditions. As such, the effects of geographical, interannual, and seasonal environmental variations could potentially be misinterpreted as stock characteristics. In theory, once the influences of environmental variables on these traits are known, this information could be used not only to discriminate stocks better, but also to identify aspects of the environmental history of fish in natural populations. Our research seeks to establish the environmental basis of variation in meristic and morphological traits and then apply the derived relationships to the inverse problem of inferring environmental history. Here we report results from the validation phase of this effort. Larvae of winter flounder, *Pseudopleuronectes americanus*, were reared from hatching to metamorphosis in the laboratory under a range of constant temperatures (7 to 16ºC). At metamorphosis, the flounder were first videotaped for size and shape measurements, then preserved, cleared and stained for enumeration of meristic traits (fin ray and vertebral numbers). We analyzed and present here the temperature dependencies of these traits as a collective suite of characteristics, but argue that meristic and morphological traits differ quantitatively in their utility to infer past environments.

Survival of Larval Winter Flounder, *Pseudopleuronectes americanus*: Evidence for a Critical Period?

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One of the dominant hypotheses in fisheries ecology is that of the 'critical period'. Since its first articulation by Hjort nearly 85 years ago, this hypothesis has been variously defined and addressed. After first reviewing the alternative definitions of critical period, we present survivorship data for laboratory populations of winter flounder, *Pseudpleuronectes americanus*, which we use to evaluate the patterns of mortality during the larval stage. Replicate populations of winter flounder were reared from hatching to metamorphosis at each of four temperatures (7 to 16ºC). Each population was monitored daily for mortality, and these data were subjected to survival ('failure time') analyses.

Overall, survival to metamorphosis was high (26 to 85%) and the survivorship curves were similar among replicates within temperature treatments. Survival was slightly lower at the cooler temperatures. For all temperatures the computed daily risk of mortality ('hazard function') was nearly uniform throughout the larval period except for older larvae where mortality increased near the ages of settlement and metamorphosis. This period of higher mortality was amplified in the populations maintained at the coolest temperature. We discuss methods to detect periods of punctuated mortality and other indicators of critical periods.

Effect of Dietary Protein Level on the Growth, Survival, and Feed Performance of Juvenile Summer Flounder, *Paralichthys dentatus*

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Protein requirement for the production of juvenile summer flounder, *Paralichthys dentatus*, was examined using experimental diets containing 45%, 55%, and 57% crude protein. Newly settled summer flounder (19 mm) were fed rations of the experimental diets and sampled for weight, length, and survival. A significant difference ($p < 0.05$) in weight occurred within the three treatments by week 3 (57>55>48). However, after six weeks, fish fed the 57% protein diet had similar growth performance (in weight) to those fed the 55 % protein diet, and were nearly significantly heavier ($p = 0.07$) than fish fed the lowest protein diet (48%). Additionally, weight-specific growth rate directly increased with protein level for the first three weeks of the experiment, but decreased among fish fed the highest protein level during the final three weeks of this experiment. Final mean values for length, weight, survival percentage, weight-specific growth rate, feed efficiency, and protein efficiency were greatest among fish fed the experimental diet containing 55% protein. These results indicate that the optimum dietary protein level for summer flounder decreases from >57% to 55% as juveniles grow from 19 mm to 50 mm. When considering the high cost of protein in manufactured feeds, we recommend a dietary protein level of 55% for juvenile (50 mm) summer flounder. Further research is needed to determine the optimum protein level for newly settled juveniles (19 mm).

Larval Rearing Techniques of Yellowtail Flounder

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Interest in yellowtail flounder, *Limanda ferruginea*, aquaculture is greatest in Atlantic Canada due to the relatively high market value and a ban on commercial fishing. Research on yellowtail flounder culture at the Ocean Sciences Centre has focused on brood-stock management, egg incubation, larval rearing and on-growing. Experiments on larval rearing were initiated in 1994 and focused on the effect of prey concentrations, prey type, light intensity and photoperiod. Results of these experiments will be discussed in terms of behavior, growth and survival.

Abstracts
Poster Presentations

Post-metamorphic Growth of Summer Flounder in Laboratory Culture: Do Early-settling Larvae Grow Faster than Late Settlers?

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Laboratory-reared summer flounder larvae begin to settle to a benthic existence 30 to 35 days after hatching, but settlement can continue for about a month because completion of metamorphosis among individuals does not occur simultaneously. We perform weekly gradings (*i.e.*, removal of settled flounder) until all fish have settled in order to prevent cannibalism and stress, because newly settled juveniles tend to be larger than swimming larvae. Although we know there is a strong correlation between larval growth and time of settlement (fastest growers settle first), no data exist on post-settlement growth variability. We wanted to determine whether fast-growing larvae become fast-growing juveniles or whether slow-growing larvae can 'catch up' in growth rate. Experiments were designed and conducted at the Narragansett Bay Campus Research Facility to explore these inquiries.

Settled fish were graded from the larval tank at 32 days after hatch (DAH) (Grade 1), 39 DAH (Grade 2), and 46 DAH (Grade 3). Graded fish were placed randomly in three replicate 75-L aquaria per grade, at a density of 30 fish per aquarium. Flounder were fed *Artemia* for 30 days after removal from the larval tank and then weaned onto a commercial diet. All fish were measured using Image Analysis at biweekly intervals until the fish were 95 DAH. No significant differences in post-settlement growth rate were seen among the three grades. In the final set of measurements, the fish exhibited an increase in size variation within replicates and cannibalism attacks were again causing mortality. Future experiments will continue to investigate specific growth rate variation in all stages of juvenile growth.

Effects of Photoperiod on Survival, Growth, and Pigmentation of Summer Flounder, *Paralichthys dentatus*, Larvae in Laboratory Culture

Marina Huber, Eric Moore, Neil Marcaccio, Robin Katersky, and David Bengtson

University of Rhode Island
Department of Fisheries, Animal and Veterinary Science
Kingston, RI 02881

The summer flounder represents a promising species for commercial aquaculture in the northeastern United States. In order to optimize production, the effects of various environmental parameters on biological production parameters must be studied. We investigated the effects of photoperiod on three parameters important to hatchery production: survival, growth and abnormal pigmentation. The last parameter involves incomplete pigmentation of the eyed side, including minor non-pigmented blotches to complete albinism. Flounder larvae were reared in replicate 75-L aquaria under three light regimes, 24L:OD (constant light), 16L:8D (summer conditions), 8L:4D:8L:4D (abnormal conditions to trick the fish into physiologically living two "days" in one). No significant differences in survival or growth were detected in the larvae through metamorphosis; however, after metamorphosis fish reared in constant light had significantly lower levels of abnormal pigmentation. The experiment will be repeated with an additional treatment, 8L:16 D (winter conditions).

Ontogenetic Diet Shifts of Larval and Juvenile Flatfish: Estimating Turnover Rates with Stable-Isotope Ratios

Keith L. Bosley[1], David A. Witting[2], Christopher Chambers[2], and Sam C. Wainright[1]

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Stable-isotope ratios of carbon (13C/12C) and nitrogen (15 N/14N) are widely used as indicators of the diet of animals. However, the use of stable isotopes to study ontogenetic diet shifts of larval and juvenile flatfishes depends upon having a knowledge of their turnover rates of carbon and nitrogen. In previous studies using wild-caught juvenile striped bass, *Morone saxatilis*, tautog, *Tautoga onitis*, and winter flounder, *Pseudopleuronectes americanus*, we found very rapid turnover rates of carbon (2-3 d) and less rapid turnover rates of nitrogen (4-17 d). In the present study, these rates were determined for larval winter flounder in the laboratory using the natural abundance of stable isotopes as tracers. Fish were spawned in the lab and maintained at two temperatures (13ºC and 17ºC) on a diet of rotifers of known isotopic composition from the time of first-feeding. At settlement, a subset of fish from both temperature treatments was maintained on rotifers to serve as a control, while the remaining fish were switched to a diet of *Artemia,* which was known to be isotopically distinct. Fish were subsampled at predetermined time intervals, and the rates of metabolic turnover of C and N were determined from plots of stable-isotopic composition as a function of time and weight gain.

Molecular Characterization of Ribosomal DNA from Representative Flatfishes of the US Atlantic Coast*

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Flatfishes are unique due to the asymmetry in their ocular rearrangements. Although young flatfishes are bilaterally symmetrical, their eyes migrate in adults either to dextral or sinistral cranium depending on the species or individuals. This migration of eyes has been reported to be genetically controlled in *Platicthys stellatus*, so we decided to revisit the phylogeny of closely related flatfishes representing sinistral and dextral ocular rearrangements based on ribosomal DNA (rDNA). The rDNA is known to contain both highly conserved and rapidly evolving domains, hence the rDNA characteristics of distantly as well as closely related fishes can be used to determine their relationships. This study examines whether ocular symmetry in flatfishes is independent of their phylogeny based on rDNA characteristics.

Samples of nuclear DNA were isolated from the blood cells of fishes representing Bothidae, Pleuronectidae and Soleidae and digested with several restriction endonucleases independently or in combinations. The DNA fragments thus generated were separated according to their molecular weights in agarose gels by electrophoresis, transferred to nylon membranes by Southern blotting techniques, and hybridized with digoxigenin-labeled *Xenopus laevis* rDNA probe. The size of each hybrid rDNA fragment was determined following the graphic method and a restriction enzyme map of rDNA was constructed for each sample species. These maps will be compared and analyzed using computer-assisted parsimony analysis to determine their phylogenetic relationships.

*Jointly supported by Brooklyn College of CUNY and The Richard Stockton College of NJ.

Size-specific Predation on Recently Metamorphosed Winter Flounder, *Pseudopleuronectes americanus*, and the Duration of their Vulnerability to *Crangon septemspinosa*

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The overall risk of predation and the specifics of the predation process are likely to depend on the relative body sizes of prey and their potential predators. We assessed the influence of body size of recently metamorphosed (8 to 30 mm TL) winter flounder, *Pseudopleuronectes americanus*, on their likelihood of being consumed by juvenile and adult (15 to 50 mm TL) sevenspine bay shrimp, *Crangon septemspinosa*. Using one-on-one laboratory-based predation trials, we show that the likelihood of predation on winter flounder by bay shrimp decreases as the flounder increase in size and/or when they are exposed to smaller shrimp. The size at which winter flounder became invulnerable to bay shrimp increased with increasing shrimp size, but occurred in all cases by the time winter flounder reached >25 mm TL. The time taken to reach a size refuge was inversely related to winter flounder growth rate, which, in turn, varied directly with prevailing water temperatures. In order to provide an estimate of the duration of the period of vulnerability of winter flounder to bay shrimp, we reared recently metamorphosed winter flounder under a range of constant temperatures (10 to 19ºC) and report here the degree-days of growth required to achieve a size refuge.

Temperature Effects on Age, Size, and Condition at Hatching in Windowpane, *Scophthalmus aquosus*

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Windowpane, *Scophthalmus aquosus*, is a broadly distributed bothid of coastal and inshore waters of the NW Atlantic. It spawns in both spring and autumn in southerly areas of its range, but this temporal bimodality converges to a single spawning season in the north. Temperature appears to be important in determining the timing of spawning, but little information exists on temperature effects on the early life history of windowpane. We expect temperature to be highly influential in fish development and growth rates in general, and it may be particularly important to windowpane ecology, given that young windowpane from the spring spawning event experience increasing temperatures whereas those from autumn spawning experience decreasing temperatures. Our intent in this study was to provide baseline information on temperature effects on embryonic period duration, survival to hatching, size and condition of fish at hatching and survival of yolk-sac larvae. We incubated replicated groups of windowpane eggs from laboratory crosses at multiple constant temperatures (7 to 21ºC) and checked these twice daily until hatching was complete. Once hatched, these fish were sized and their survival in the absence of food was monitored. Survival to hatching was highest at the intermediate temperatures. The duration of the embryonic period ranged from 2 to over 11 d, and varied inversely with incubation temperature. The intermediate incubation temperatures resulted in the largest larvae at hatching. Duration of the yolk-sac period of larvae was independent of the temperature experienced during their embryonic periods. Our results are compared with those from other *Scophthalmus* species and with marine teleosts in general.

The Distribution and Size Composition of Five Flatfish Species in Long Island Sound Based on the Connecticut Fisheries Division Bottom-trawl Survey, 1984-1994

Kurt F. Gottschall, Mark W. Johnson, and David G. Simpson

Connecticut Department of Environmental Protection
Fisheries Division
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The distribution, abundance and length composition of five flatfish species in Long Island Sound-fourspot flounder (*Paralichthys oblongus*), hogchoker (*Trinectes maculatus*), summer flounder (*Paralichthys dentatus*), windowpane flounder (*Scophthalmus aquosus*), and winter flounder (*Pseudeopleuronectes americanus*) are examined relative to season and physical features of the Sound using Connecticut Department of Environmental Protection bottom-trawl survey data collected from 1984 to 1994. Catches were plotted on maps of the Sound to display preferences for particular areas. Four species were mapped by season, while winter flounder were mapped by month and two size groups to show seasonal and spatial patterns by size. An overall length frequency was prepared for each species to show the size classes sampled by the Survey. Abundance indices were calculated and plotted by month, month and bottom type, and month and depth interval. Overall preferences for depth interval and bottom type were tested by ANOVA. Seasonal migration patterns, preferences for particular areas, and preferences for depth and bottom types within Long Island Sound are evident for all five species. For summer flounder, windowpane, and winter flounder, which are currently managed by fishery management plans, these results may be useful for the determination of essential fish habitat as required by the federal Magnuson-Stevens Act.

Aspects of the Life History of Hogchoker, *Trinectes maculatus*, in Delaware Bay Marsh Creeks

Ralph Bush and Kenneth Able

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During 1996 and 1997 we sampled in intertidal and subtidal habitats with weirs (n =110) and otter trawls (n =1137) to determine aspects of the life history of hogchoker, *Trinectes maculatus*. Over the salinity range sampled (0-16 ppt), hogchokers appear most dominant in creeks at intermediate salinities where they were dominated by 50-100 mm TL individuals which are approximately 1-2 years old based on a study conducted by Mansueti and Pauly (1956). In one creek system (Madhorse Creek, 10-12 ppt range), in which they were a dominant component of the fish fauna, the distribution varied with habitat type and season. They seldom occurred on the intertidal marsh surface, were slightly more abundant in shallow subtidal creeks in MLW, and most abundant in deeper 2-3 m subtidal creeks. We are currently examining aspects of reproduction and the distribution and abundance of YOY hogchokers along this salinity gradient.

Growth of Young-of-the-Year Winter Flounder, *Pseudopleuronectes americanus*, within Eelgrass, *Zostera marina*: Impact of Habitat Edge

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Winter flounder, *Pseudopleuronectes americanus*, recruit to shallow estuarine habitats throughout their distribution. In an attempt to assess the relative value of these habitats, growth of young-of-the-year were measured in Little Egg Harbor, NJ in May and June of 1998. Comparative growth (*i.e.*, changes in standard length [mm] and weight [g]) was assessed through caging experiments in unvegetated habitat and from edge and interior portions of eelgrass, *Zostera marina*. Three caging experiments were undertaken using four replicate cages in each habitat, each containing three marked fish. Fish growth was expressed as G day^{-1} to eliminate size bias of fish differing in initial lengths and weights. Results from experimental trials indicate that *P. americanus* had greater mean G_{length} values in unvegetated habitat (0.016 day^{-1}) compared to *Z. marina* edge (0.014 day^{-1}) and interior (0.011 day^{-1}), as well as greater G_{weight} values (0.061, 0.06, and 0.047 day^{-1}; unvegetated, edge, and interior, respectively). Additionally, growth rates declined during each successive caging trial, with growth significantly reduced by the end of June ($P < 0.0001$). Temperature data collected during the experiments suggest a possible inverse relationship between growth and increasing temperature. Benthic core samples were also collected to assess the distribution of potential prey greater than 250ìm (*e.g.*, amphipods, polychaetes). Preliminary results from May showed that potential prey density was greatest at *Z. marina* edge (155,911 m^{-2}), reduced in interior *Z. marina* (115,124 m^{-2}), and significantly lower from samples gathered in unvegetated sand (42, 102 m^{-2}). These data provide evidence that although potential prey density was less in unvegetated habitats, growth of *P. americanus* was greater there. This suggests that feeding by *P. americanus* may be impeded in *Z. marina*.

An Evaluation of the Relationship between Otolith Microstructure, Otolith Growth, and Somatic Growth in a Temperate Flatfish, *Scophthalmus aquosus*

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We examined the value of otolith microstructure for interpreting growth during the first year of life of windowpane, *Scophthalmus aquosus*, a northwest Atlantic bothid that exhibits bimodal (spring and fall) spawning behavior. Laboratory analysis of sagittal otoliths marked with oxytetracycline (0.9 $g*l^{-1}$) supported the view that otolith increments are indicative of daily age for spring-spawned individuals (n=45, 15-97 mm TL) held under summer conditions. We were unable to resolve daily increments for spring-and fall-spawned individuals (n=16, 82-140 mm TL and n=27, 15-37 mm TL, respectively) held under winter conditions. When data from the three experimental groups (spring-spawned summer growth, spring-spawned overwinter growth, and fall-spawned overwinter growth) were examined separately, we detected differences in the otolith-somatic size and growth relationships between cohorts. When these data were pooled, we identified a significant decrease in the relationship between otolith size and somatic size for individuals > 57 mm TL. Our results emphasize that size-based ontogenetic state, cohort and growth conditions need to be considered when utilizing otolith sizes or inter-increment distances for back-calculating somatic sizes and growth rates.

Development of Spawning and Rearing Techniques for Southern Flounder in South Carolina

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The southern flounder, *Paralichthys lethostigma*, appears to be an excellent candidate for commercial culture in the southern United States. However, controlled spawning techniques have not been developed. Several strip-and tank-spawning experiments were conducted using previously spawned wild flounder held in captivity for two years. Fish were photo-thermally conditioned to spawn 3 months after the natural season. For the specific spawning trials, females with oocytes >500 µm were selected. Larval rearing experiments using commercial larval fish diet and combinations of enriched and unenriched rotifers and *Artemia* were conducted on offspring produced from these trials.

In the strip-spawning study 3 treatments were examined: 1) use of 100 µg GnRHa implants; 2) re-implantation of fish which ceased to spawn; and 3) natural ovulation. Mean egg production of implanted fish (mean size 1.48 kg) was > 376,250 eggs/female and fertility was 69.5%. These fish began ovulating within 48 hr post-hormone treatment and spawned ~3 times. Naturally ovulating fish (mean size 1.38 kg) produced 645,000 eggs/female, however fertility was only 37.6%. Mean spawning frequency (n=7) was significantly higher than for the hormone-induced fish. Re-implanted females also produced eggs (208,000 eggs/female) within 48 h, but fertility (39%) was significantly less than that recorded from the first spawn after initial implantation.

A number of tank spawnings were conducted using females > 2.0 kg in weight. In all studies, a 2:1 male to female sex ratio was maintained. The first study compared implanted (100 µg GnRHa) *vs* nonimplanted females placed in tanks with nonhormone treated males. Fertilized eggs were regularly produced from the tank containing the implanted females while only nonfertilized eggs were obtained from the tank containing the nonimplanted females. The second study used testosterone implants to examine their potential to elicit courtship and spawning behavior among nonperforming males. Courtship behavior was not noted and only nonfertilized eggs were produced. In the third study, the females from study 2 were hormonally-treated (100 µg GnRHa) and returned to the same group of males. Several groups of fertilized eggs were produced but then spawning ceased. The final study examined the potential of spawning small females (< 1.5 kg) implanted with 100 ìg GnRHa. Several small batches of fertilized eggs were produced initially but spawning ceased shortly thereafter.

Our results indicate that fertilized eggs can be produced by strip-spawning naturally ovulating females and spermiating males. GnRHa implants can hasten ovulation but duration of spawning is shorter than among naturally ovulating females. Reimplantation of spawned females can result in production of additional eggs but number of eggs and fertility are lower. Male courtship behavior appears to be the limiting factor in production of fertilized eggs from tank spawning. Use of GnRHa-implanted females has been helpful in stimulating male courtship.

A larval rearing study was designed to determine if survival and pigmentation is affected by diet combinations of rotifers and *Artemia* (enriched and unenriched) and Lansy diet. At 24ºC, five-day-old larvae (1.98 ± 0.1 mm TL) began feeding on the rotifer *Brachionus plicatilis* and completed metamorphosis by day 30. In treatment 1, larvae (6 dph) were fed rotifers (unenriched)(10/ml) days 6-15 and *Artemia* nauplii (unenriched)(3/ml) day 7 through metamorphosis. The second treatment was fed rotifers (HUFA-enriched) from 6 dph through metamorphosis and *Artemia* (HUFA-enriched) 7 dph through metamorphosis, while

treatment 3 was fed rotifers (unenriched)(10/ml) days 6-15, *Artemia* (HUFA-enriched) 7 dph through metamorphosis and a commercially prepared larval diet (Lansy diet) day 13 through metamorphosis. Survival was variable and no differences were detected between treatments. Treatment 3 had significantly more normally pigmented fish than did any of the other treatments.

Comparison of Diets Among Four Co-occurring Juvenile Flatfishes near Kodiak Island, Alaska

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Diets of four abundant juvenile flatfishes near Kodiak Island, Alaska were evaluated with respect to size of predator, and the depth and substrate of capture. Age-0 and age-1 flathead sole, *Hippoglossides elassodon*, Pacific halibut, *Hippoglossus stenolepis*, yellowfin sole, *Pleuronectes asper*, and rock sole, *Pleuronectes bilineatus*, were collected at depths ranging from 0 to 100 m and on substrates of mud, sand, or gravel. All fishes primarily consumed small crustaceans, of which the most common taxa were mysids, crustaceans, and gammarid amphipods. The specific prey taxa consumed depended on the size of predator and the physical parameters of the capture site. There were indications that interspecific and intraspecific dietary overlap were reduced at sites where predators of different species or different sizes within a single species were captured together.

Somatic Growth and Otolith Growth in Juvenile Fringed Flounder, *Etropus crossotus*

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The fringed flounder, *Etropus crossotus*, in South Carolina has a maximum life span of 1.5 years and spawns from March through October. The long spawning period makes it difficult to determine growth rates of individuals and the population as a whole. Growth rates can only be estimated if the size and age of field-collected individuals is known with precision. A laboratory growth experiment was designed to estimate short-term growth rates of juvenile fringed flounder at various temperatures, and to investigate the relationship between otolith growth and somatic growth. Growth of the fish was determined with Alizarin-marked fish at 14, 19, 24 and 28ºC, under defined feeding conditions for 66 days. After an adaptation period of 18 days, the fish were measured and weighed at 12-day intervals. The number of daily increments formed during the experiment was not significantly different from 66, validating formation of 1 increment/day. The somatic growth increased with temperature and was highest at 24 and 29ºC. The width of increments formed during the experiment increased with increasing somatic growth. The nature of this relationship and its use for estimating growth of individuals from field collections will be discussed.

Utilization of Intertidal and Marina Habitats by Juvenile Winter Flounder, *Pseudopleuronectes americanus*

Matthew E. Mroczka[1], John K. Carlson[2], Todd A. Randall[3], and Peter E. Pellegrino[4]

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Creation of marinas involves removal of integral parts of the existing ecosystem such as salt marshes and intertidal flats which function as nursery habitat for juvenile fish. Yet, preliminary studies on marinas have suggested that they do not totally displace juvenile fish. To evaluate this suggestion, the relative abundance of juvenile winter flounder, *Pseudopleuronectes americanus*, was compared in two areas: a marina basin and an adjacent intertidal habitat. Winter flounder were sampled with a 1-meter beam trawl monthly from March through November 1990-1995. Both habitats were dominated by young-of-the-year and age 1+ fish. We found no significant difference in the relative abundance of flounder among habitats for all combined years sampled. The average density was 0.04 ± 0.06 flounder/m within the marina and 0.03± 0.04 flounder/m within the intertidal flat. We found seasonal variation in abundance with highest number caught during the summer months (June-August) and lowest during spring (March-May). The results of this study suggest that young-of-the-year winter flounder are equally abundant in both natural intertidal habitats and marina basins, indicating that both could serve as nurseries. However, more specific research is required to resolve the importance of marinas and the factors involved in the utilization of each habitat.

Winter Flounder Tagging in Western Cape Cod Bay in the Decade of the 1990s: Movements, Fidelity, and Population Size

R. Lawton, B. Kelly, J. Boardman, and V. Malkoski

Massachusetts Division of Marine Fisheries, Pocasset, MA 02559

No Abstract Available

Effects of 2, 3, 7, 8- Tetrachloroolibenzo-p-Dioxin on Winter Flounder Embryos from NY/NJ Harbor Estuary and Long Island Sound

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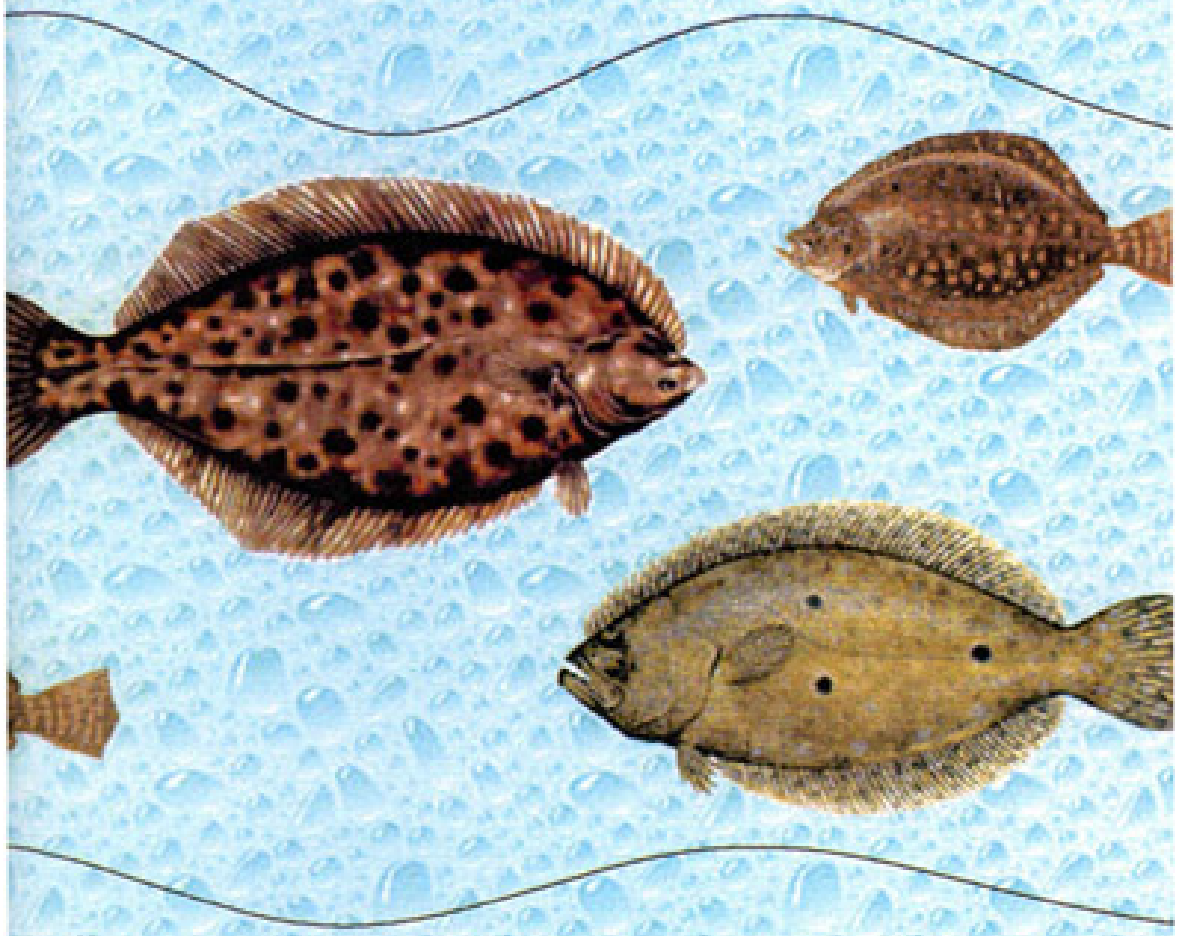
No Abstract Available

Flatfish Biology Conference



December 5-6, 2000

Mystic, Connecticut

Northeast Fisheries Science Center Reference Documents

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Flatfish Biology Workshop
December 5-6, 2000, Mystic, Connecticut

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Seventh in a series of Flatfish Biology Conferences

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Flatfish Biology Workshop

December 5-6, 2000, Best Western Sovereign Hotel, Mystic, Connecticut

Oral Presentations

Tuesday, December 5th

8:00 a.m. **Registration/Coffee, Danish and Muffins**

8:45 a.m. Welcome and Introduction
Anthony Calabrese, Chair
National Marine Fisheries Service
Milford Laboratory
Milford, CT

John Boreman, Director
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, MA

Session I

Penny Howell, Chair
Connecticut Department of Environmental Protection
Fisheries Division, Old Lyme, CT

9:00 a.m. Beyond Winter Flounder: A Global Perspective of Species Diversity in the Flatfish (Order Pleuroneciformes)
Thomas Munroe
National Marine Fisheries Service, National Systematics Laboratory, Washington, DC

9:20 a.m. Melanophore Receptor Diversity as a Component of Flatfish Patterning Physiology
Derek Burton, Dennis Mayo, and Joanne Vokey
Memorial University of Newfoundland, Department of Biology, St. John's, Newfoundland, Canada

9:40 a.m. Pigmentation Development in Hatchery-reared Summer Flounder
Jessica Bolker
University of New Hampshire, Department of Zoology, Durham, NH

10:00 a.m. Patterns of Reproduction in Flatfish: Frequency, Up-regulation and Down-regulation
Margaret Burton
Memorial University of Newfoundland, Department of Biology, St. John's, Newfoundland, Canada

10:20 a.m. **Coffee/Danish/Muffins**

Session II

Don Danila, Chair
Northeast Utilities Environmental Laboratory
Waterford, CT

10:50 a.m. Experimental Analysis of Geographic and Seasonal Differences in Spawning Time on Growth, Development, and Survival of Larval Summer Flounder, *Paralichthys dentatus*
Chris Chambers and David Witting
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

11:10 a.m. Timing of Feeding Transition and Individual Consumption Rates Affect Growth of Summer Flounder (*Paralichthys dentatus*) Larvae
Markus Koelbl and David Bengtson
University of Rhode Island, Department of Fisheries, Animal and Veterinary Sciences, Kingston, RI

11:30 a.m. Energetics of Growth Differences in Cultured Summer Flounder, *Paralichthys dentatus*: Prey Consumption and Absorption Efficiency by Newly-settled Juveniles
Tessa Simlick[1], Robin Katersky[1, 2], and David Bengtson[1]
[1]University of Rhode Island, Department of Fisheries, Animal and Veterinary Sciences, Kingston, RI, and [2]National Marine Fisheries Service, Milford Laboratory, Milford, CT

11:50 a.m. The Oxygen Consumption, Ammonia Excretion and Scope for Growth of Newly Settled Summer Flounder, *Paralichthys dentatus*
Robin Katersky[1, 2], Tessa Simlick[2], and David Bengtson[2]
[1]National Marine Fisheries Service, Milford Laboratory, Milford, CT, and [2]University of Rhode Island, Department of Fisheries, Animal and Veterinary Sciences, Kingston, RI

12:10 p.m. **Hosted Buffet Lunch**

Session III

Ambrose Jearld, Chair
National Marine Fisheries Service
Woods Hole, MA

1:10 p.m. Correlations among Stage-specific Abundances of Larval and Juvenile Winter Flounder near Millstone Point, CT and their Relation to Year-class Strength
Donald Danila[1], Ernest Lorda[1], and J. Dale Miller[1, 2]
[1]Northeast Utilities Environmental Laboratory, Waterford, CT and [2]Normandeau Associates, Plymouth, MA

1:30 p.m. Distribution, Life History, and Identification of Southern U. S. *Paralichthys* with Implications for Management
Bruce Thompson and Andrew Fischer
Louisiana State University, Coastal Fisheries Institute, Baton Rouge, LA

1:50 p.m. Abundance, Density and Life Frequency of Juvenile English Sole in Pacific Coast Estuaries from 1998-2000
Christopher Rooper and Donald Gunderson
University of Washington, School of Fisheries, Seattle, WA

2:10 p.m. The Distribution and Abundance of Juvenile American Plaice, *Hippolossoides platessoides*, in the Southwestern Gulf of Maine, New Hampshire
Augustine Mungkaje and W. Huntting Howell
University of New Hampshire, Zoology Department, Durham, NH

2:30 p.m. Plasticity in Size, Age and Development of at Settlement Individually Reared Winter Flounder (*Pseudopleuronectes americanus)* Larvae
Marc Gravel and Joseph Brown
Memorial University of Newfoundland, Ocean Sciences Centre, St. John's, Newfoundland, Canada

2:50 p.m. **Refreshment Break**

Session IV

Chris Powell, Chair
Rhode Island Division of Environmental Management
Wickford, RI

3:20 p.m. Temperature Effects on Embryonic Developmental Rates, Size at Hatching, and Survival of Yolk-sac Larvae of Summer Flounder, *Paralichthys dentatus*
Peter Berrien, John Sibunka, and Chris Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

3:40 p.m. Metamorphosis in Summer Flounder: Manipulation of Thyroid Status to Modify Growth, Development and Settling Behavior
Steven Gavlik and Jennifer Specker
University of Rhode Island, Graduate School of Oceanography, Narragansett, RI

4:00 p.m. Ontogenetic Transitions During the First Year of Life in Two Cohorts of Windowpane Flounder, *Scophthalmus aquosus*
Melissa Newman and Ken Able
Rutgers University Marine Field Station,Tuckerton, NJ

4:20 p.m. Gonadotropin-releasing Hormone Affects Sperm Production of Atlantic Halibut, *Hippoglossus hippoglossus*
Debbie Martin-Robichaud[1] and J. Powell[2]
[1]Fisheries and Oceans, Biological Station, St. Andrews, New Brunswick, Canada, and [2]Syndel International, Inc., Vancouver, British Columbia, Canada

4:40 p.m. Larval Winter Flounder Stock Identification using Microelements: First-year Analysis (2000) and Preliminary Results
Saul Saila[1], Ernest Lorda[2], Bradley Moran[1], and Donald Danila[2]
[1]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI, and [2]Northeast Utilities Environmental Laboratory, Waterford, CT

5:00 p.m. **Poster set-up**

6:00 p.m. **Hosted Mixer and Poster Session**

Wednesday, December 6th

7:45 a.m. **Registration/Coffee, Danish and Muffins**

Session V

Jeremy King, Chair
Massachusetts Division of Marine Fisheries
Pocasset, MA

8:20 a.m. Winter Flounder, *Pseudopleuronectes americanus*, Stock Enhancement in New Hampshire: Evaluating Release Locations
Elizabeth Fairchild and W. Huntting Howell
University of New Hampshire, Zoology Department, Durham, NH

8:40 a.m. Effects of Scallop Dredging on a Recently Settled Continental Shelf Flatfish: Disturbance or Status Quo?
Mark Sullivan[1], Robert Cowan[1], Kenneth Able[2], and Michael Fahay[3]
[1]Rosenstiel School of Marine and Atmospheric Science, Division of Marine Biology and Fisheries, Miami, FL., [2]Rutgers University, Marine Field Station, Tuckerton, NJ, and [3]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

9:00 a.m. Dynamic Habitat in a Temperate Estuary and Spatio-temporal Variation in the Growth of an Early Juvenile Flatfish
John Manderson[1], Beth Phelan[1], Carol Meise[1], Linda Stehlik[1], Allen Bejda[1], Jeff Pessuti[1], Linda Arlen[1], Andrew Draxler[1], and Allan Stoner[2]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [2]National Marine Fisheries Service, Hatfield Marine Sciences Laboratory, Newport, OR

9:20 a.m. Recruitment of Summer Flounder Larvae to Chesapeake Bay: Larval Flux at an Inlet
Harvey Walsh[1], Jonathan Hare[1], Simon Thorrold[2], Arnoldo Valle-Levinson[3], Chris Reiss[3], and Cynthia Jones[3]
[1]NOAA, National Ocean Service, Center for Coastal Fisheries and Habitat Research, Beaufort Laboratory, Beaufort, NC, [2]Old Dominion University, Department of Biological Science, Norfolk, VA, and [3]Old Dominion University, Ocean, Earth, and Atmospheric Sciences, Norfolk, VA

9:40 a.m. Use of A Rhode Island Salt Pond by Juvenile Winter Flounder, *Pseudopleuronectes americanus*
Maureen Koprowski, Chris Orphanides, Marnita Chintala, Giancarlo Cicchetti, and Lesa Meng
U.S. Environmental Protection Agency, Office of Research and Development, Atlantic Ecology Division, Narragansett, RI

10:00 a.m. **Coffee/Danish/Muffins**

Session VI

Jay Burnett, Chair
National Marine Fisheries Service
Woods Hole, MA

10:20 a.m. Experimental Evaluation of Ontogenetic Diet Transitions in Summer Flounder, *Paralichthys dentatus*, Using Stable Isotopes as Diet Tracers
David Witting[1], Keith Bosley[2], Christopher Chambers[1], and Sam Wainright[3]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, [2]National Marine Fisheries Service, Hatfield Marine Sciences Laboratory, Newport, OR, and [3]U. S. Coast Guard Academy, Department of Science, Academic Division, New London, CT

10:40 a.m. Restriction Endonuclease Characters of Flatfish Ribosomal DNA may Help Resolve their Phylogeny
Z. M. G. Sarwar. Jahangir[1], Ronald Eckhardt[2], and Pradip Kar[2]
[1]Wabash College, Department of Biology, Crawfordsville, IN, and [2]Brooklyn College of the City University of New York, Department of Biology, Brooklyn, NY

11:00 a.m. An Examination of Winter Flounder (*Pseudopleuronectes americanus*) Larvae Genetic Stock Structure in Long Island Sound
Joseph Crivello[1], J. Dale Miller[2, 3], Donald Danila[2], Milan Keser[2], Ernest Lorda[2], and Saul Saila[4]
[1]University of Connecticut, Department of Physiology and Neurobiology, Storrs, CT, [2]Northeast Utilities Environmental Laboratory, Waterford, CT, [3]Normandeau Associates, Plymouth, MA, and [4]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI

11:20 a.m. The Role of Carbonic Anhydrase in Renal Sulfate Secretion by Winter Flounder (*Pseudopleuronectes americanus*)
Larry Renfro[1, 2], Thomas Maren[2], Eric Swensen[2], David Miller[2], and Alice Villalobos[2]
[1]University of Connecticut, Department of Physiology and Neurobiology, Storrs, CT, and [2]Mount Desert Island Biological Laboratory, Salisbury Cove, ME

11:40 a.m. The Effects of Hypoxia on Growth and Hematology of Juvenile Summer and Winter Flounder
Kevin Stierhoff and Timothy Targett
University of Delaware, College of Marine Studies, Lewes, DE

12:00 p.m. **Hosted Buffet Lunch**

Session VII

Renee Mercaldo-Allen, Chair
National Marine Fisheries Service
Milford, CT

1:10 p.m. Foraging in Juvenile Summer and Southern Flounder: Effects of Light, Turbidity and Prey Type
Ursala Howson[1, 2] and Timothy Targett[2]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [2]University of Delaware, College of Marine Studies, Lewes, DE

1:30 p.m. An Experimental Analysis of Size-specific Predator-prey Interactions between Juveniles of Summer Flounder, *Paralichthys dentatus*, and Winter Flounder, *Pseudopleuronectes americanus*
Chris Chambers and David Witting
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

1:50 p.m. Response of YOY Winter Flounder to Sediment Biogeochemicals
Andrew Draxler[1] and Jessica Siclare[2]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [2]Saint Joseph's University, Philadelphia, PA

2:10 p.m. Field and Laboratory Observations on Feeding Behavior of Newly Settled Winter Flounder, *Pseudopleuronectes americanus*
Patricia Shaheen[1], Linda Stehlik[2], Carol Meise[2], Allan Stoner[3], John Manderson[2], and Danielle Adams[2]
[1]Rutgers University, Institute of Marine and Coastal Sciences, New Brunswick, NJ, [2]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [3]National Marine Fisheries Service, Hatfield Marine Sciences Laboratory, Newport, OR

2:30 p.m. GIS Mapping of Winter Flounder (*Pseudopleuronectes americanus*) Data for Rhode Island Waters, an Effort to Identify Essential Fish Habitat (EFH)
Wilfrid Rodriguez[1], Peter August[1], and Chris Powell[2]
[1]University of Rhode Island, Environmental Data Center, Kingston, RI and [2]Rhode Island Division of Fish and Wildlife, Marine Fisheries, Wickford, RI

2:50 p.m. **Wrap-up**

3:00 p.m. **Adjourn**

Poster Session

Tuesday, December 5th, 6:00 p.m.

Comparison between Two Methodologies for Batch-marking Adult Winter Flounder: Preliminary Results
Donald Danila
Northeast Utilities Environmental Laboratory, Waterford, CT

A Family of Pleurocidin-like Antimicrobial Peptides from Winter Flounder
Jeffery Gallant and Susan Douglass
Institute for Marine Biosciences, Halifax, Nova Scotia

Spatial Distribution of Flounder Collected in Channel and Shoal Habitats of the New York and New Jersey Harbor Estuary as Related to Sediment Characteristics
Teresa Nelson[1], John Duschang[1], and Jenine Gallo[2]
[1]LMS Engineers LLP, Pearl River, NY, and [2]U. S. Army Corps of Engineers-NY District New York, NY

Increase in Numbers of Smallmouth Flounder, *Etropus microstomus*, in the Ichthyoplankton of Narragansett Bay and Mount Hope Bay, RI
Grace Klein-MacPhee[1], Michael Scherer[2], Richard Satchwill[3], Aimee Keller[1], and Carol Vasconcelas[3]
[1]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI, [2]Marine Research, Falmouth, MA, and [3]Rhode Island Department of Environmental Management, Coastal Fisheries Laboratory, Wakefield, RI

GIS Mapping of Winter Flounder (*Pseudopleuronectes americanus*) Data for Rhode Island Waters, an Effort to Identify Essential Fish Habitat (EFH)
Wilfrid Rodriguez[1], Peter August[1], and Chris Powell[2]
[1]University of Rhode Island, Environmental Data Center, Kingston, RI, and [2]Rhode Island Division of Fish and Wildlife, Marine Fisheries, Wickford, RI

Density-dependent Changes in Area of Habitat Occupied by Georges Bank Yellowtail Flounder (*Limanda ferruginea*)
Travis Shepherd and Matthew K. Litvak
University of New Brunswick, Saint John, New Brunswick, Canada

Abstracts
Oral Presentations

Beyond Winter Flounder: A Global Perspective of Species Diversity in the Flatfish (Order Pleuronectiformes)

Thomas A. Munroe

National Marine Fisheries Service, National Systematics Laboratory
National Museum of Natural History
Washington, DC 20560

More than 690 species of flatfish are currently recognized worldwide; with species distributed from northern polar seas to southern boreal seas. Most commercially important flatfish occur in northern oceans, however, the greatest diversity of flatfish species (ca. 466 species, 68%+ of total diversity) is in tropical shallow waters. Systematic knowledge for most commercially important flatfishes in northern waters is well known, however, for most other flatfishes this is not the case. For the Pleuronectidae (60 species, 9% of total diversity) and Scophthalmidae (8 species) and tropical families with low diversity, such as the Psettodidae (2 species) and Citharidae (6 species), we have a good working knowledge of species-level diversity. New species are still being described in Paralichthyidae (94 species, 14% of total diversity) and Rhombosoleidae, although rates of new descriptions for these families suggest we are also approaching a good understanding regarding taxonomy of these species. For Cynoglossidae (142 species, 21% of species diversity), Bothidae (138 species, 20% of total diversity), Soleidae (139 species, 20% of total diversity) and Achiridae (ca. 35 species, 5% of diversity), many new species have been described recently and more work is needed to properly assess species diversity in these families. From a global perspective, north temperate and polar species number about 115 (ca. 17% of total diversity), with few new species being discovered in these areas. Flatfish diversity in southern temperate and subantarctic waters is low (only 4% of diversity), and the majority of species were described in the past 50 years. Freshwater flatfishes (only 2% of total diversity) are relatively rare, but undescribed species, especially in South America, continue to be discovered. New species are still being discovered at a relatively high rate in tropic waters. The majority of flatfishes are 30 cm or less in adult size. Most new species described in the last 50 years are 20 cm or less in length, suggesting that probably no large-sized species of flatfishes remain to be discovered. Present estimates are that ca. 36% of flatfish species occur in neritic and shallow water habitats, 272 species (39% of total diversity) inhabit inner continental shelf habitats; 153 species (22%) live on the outer shelf and upper slope; and 18 species (3%) occur only on the continental slope. The greatest levels of flatfish diversity occur on the inner continental shelf and in neritic habitats in 100 m or less. Nearly 24 % of the total known species have or will be described from 1951 to the present. That 24% of the total known species of flatfishes has been described only within the past 50 years indicates that we are not yet close to knowing the total diversity of species in the Pleuronectiformes.

Melanophore Receptor Diversity as a Component of Flatfish Patterning Physiology

Derek Burton, Dennis Mayo, and Joanne E. Vokey

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Winter flounder (*Pseudopleuronectes americanus*) can display a chromatophore pattern with white spots, dark bands, and general background as major components. Melanophores from these major pattern components show variable responses to background changes and to stress, and they are predominantly under adrenergic neural control. *In vitro* experiments with catecholamines and their antagonists, as well as with melanophore stimulating hormone (MSH) and melanophore concentrating hormone (MCH), can demonstrate pattern-related differences in melanosome aggregative and dispersive responsiveness. Such experiments indicate that α_1- and α_2- adrenoceptors, and also MCH receptors, mediate melanosome aggregation and that β_1- and β_2- adrenoceptors, as well as MSH receptors, are involved in melanosome dispersion. These results demonstrate that melanophore receptor diversity is an important facet of flatfish patterning physiology facilitating subtle gradations in balance between those which are antagonistic and synergistic in their effect.

Pigmentation Development in Hatchery-reared Summer Flounder

Jessica A. Bolker

University of New Hampshire
Department of Zoology
208 Rudman Hall, 46 College Road
Durham, NH 03824

Malpigmentation is a common problem in hatchery-reared flatfishes, and diminishes both their market value and their suitability for stock enhancement. Although much progress has been made in preventing malpigmentation through supplementation of larval diets, the fundamental causes of pigmentation problems and the mechanisms of pigmentation development, remain obscure. I examined the appearance and distribution of pigment cells in summer flounder (*Paralichthys dentatus*) larvae from hatching through metamorphosis, using a variety of microscopical techniques. Melanophores and xanthophores appear early in development, and reflective iridophores differentiate later. After metamorphosis, the blind side of normal fish is populated almost exclusively by iridophores; these cells are present on the ocular side as well, but largely masked by melanophores. Abnormally-pigmented light patches on the ocular side of postmetamorphic juveniles closely resemble normal blind-side skin, and dark areas on the blind side appear much like normal ocular-side skin. This pattern suggests that some forms of malpigmentation may be due to spatially inappropriate activation of normal developmental processes.

Patterns of Reproduction in Flatfish: Frequency, Up-regulation, and Down-regulation

Margaret P. M. Burton

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Initial studies on winter flounder, *Pseudopleuronectes* (*Pleuronectes) americanus*, showed that this flatfish, off Newfoundland, had a very short spawning time for individual females unlike other locally occurring pleuronectids such as yellowtail, *Limanda ferruginea* (*Pleuronectes ferruginea*), or American plaice, *Hipploglossoides platessoides*, which are batch or serial spawners, and which have the possibility of up-regulation by very late recruitment of oocytes after spawning has begun. If this is a widespread option for "group synchronous" batch-spawners, then the idea of determinacy for fecundity studies prior to spawning may need some adjustment. Studies on winter flounder indicated that wild fish, both males and females, were subject to spawning omission, itself probably related to the long winter fast off Newfoundland, and it is still not clear how widespread this type of irregular spawning pattern may be; it is more difficult to recognize in batch-spawning fish with a lengthy spawning season. However, records indicate such spawning omission for halibut (at high levels) and for American plaice off Newfoundland. Recent studies on groundfish, including flatfish, off Newfoundland have shown some populations with high levels of atresia during late vitellogenesis, producing a large amount of down-regulation, perhaps even two years of reproductive omission, in females. It is not known why this is occurring or whether it is a new phenomenon; it does not seem to have been reported before for flatfish, except in the Barents Sea for *Reinhardtius hippoglossoides*, in connection with temperature changes, and in the Western English Channel for *Pleuronectes platessa*, in an area previously contaminated by the oil-spill from the Amoco Cadiz.

Experimental Analysis of Geographic and Seasonal Differences in Spawning Time on Growth, Development and Survival of Larval Summer Flounder, *Paralichthys dentatus*

R. Christopher Chambers and David A. Witting

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Summer flounder, *Paralichthys dentatus*, is a widely distributed paralichtyid of subtropical and temperate waters of the U. S. Atlantic Coast. Egg frequencies in ichthyoplankton collections suggest that spawning occurs from southern Georges Bank to North Carolina in the autumn, initiating earlier in the north than the south. Eggs and larvae are abundant in waters of southern New England and the New York Bight. In contrast, young juvenile summer flounder are infrequent in these northern areas relative to their abundance in the bays of the Carolinas and Virginia. It may be surmised that eggs and larvae spawned in northern areas are largely lost to recruitment because of cooler water temperatures there. This experimental study was designed to elucidate the effects of autumn through spring water temperatures on summer flounder larvae. Using a 2^2- factorial design, we evaluated the effects of early versus late spawning times and northern versus southern water temperature regimes on the growth, development and survival of summer flounder larvae. In addition, we challenged larvae of various sizes (6 to 10 mm TL) to cooler temperatures typical of inner shelf conditions in winter as larvae might encounter while migrating shoreward. We also challenged late-staged, transitional larvae (12 to 14 mm TL) to temperatures typical of bays and estuaries in winter as these larvae might experience during ingress. Larvae that experienced temperatures intended to simulate spawning at southerly latitudes, and larvae reared at temperatures mimicking a spawning origin in early autumn at northerly latitudes, reached the developmental stages associated with ingress before cool winter temperatures prevented further development. Larvae with the shortest initial season of growth (*i.e.*, products of northerly spawning in late autumn) failed to reach ingress stages until temperatures warmed in spring, but by this time these larvae were significantly larger-at-stage than were members of cohorts that ingressed before winter. We discuss these results, along with those on inner-shelf and ingress challenges, in the context of a temperature-based habitat map for the early life history stages of summer flounder.

Timing of Feeding Transition and Individual Consumption Rates Affect Growth of Summer Flounder (*Paralichthys dentatus*) Larvae

Markus Koelbl and David A. Bengtson

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Minimizing inter-individual variation in the growth and size of cultured fish is necessary for the synchronization of fish to a target size, reducing competitive interactions and cannibalism, reducing food wastage, and improving production efficiency. We investigated the causes of size variation in larval summer flounder that magnifies around 20-25 days after hatch (DAH), several days after the larvae make the transition from rotifers to *Artemia* nauplaii. Specifically, we conducted two experiments to determine whether the time of transition to *Artemia* and consumption rates of *Artemia* by individual larvae may contribute to varying growth rates. In the first experiment we examined size differences between larvae that could ingest *Artemia vs*. those who could not on days 12, 13, 14, and 15 DAH and grew replicate batches of each group to 30 DAH to determine if day of feeding transition was related to size at 30 DAH. In the second experiment, we monitored daily *Artemia* consumption rates of individual larvae that made the rotifer- *Artemia* transition at 12 *vs* 13 *vs* 14 DAH and related that to size at 26 DAH. The first experiment showed that the larva's ability to transition from rotifers to *Artemia* is size-dependent, but also that larvae making the transition at 13 DAH grew significantly larger than their siblings making the transition at 12 or 15 DAH. The second experiment showed a high degree of variability in inter-individual consumption patterns. Larval size at 26 DAH correlated well with food consumption by individuals, with consumption explaining 72-93% of the variance in fish size. No significant differences were found in the food consumption-fish size relationship for larvae that made the rotifer- *Artemia* transition on 12 *vs* 13 *vs* 14 DAH.ANOVA showed that ability to ingest *Artemia* at 12 DAH did not provide a consumption advantage over larvae that started eating *Artemia* a day or two later. An examination of the correlations between fish size at 26 DAH and cumulative food consumption through days 17, 20, or 23, showed a greatly increased correlation between day 17 and 20. Based on the results of both experiments, we conclude that there is no growth advantage for fish that can feed on *Artemia* earlier than their siblings (although there may be an advantage for the transition on 13 DAH). The second experiment showed that there is a critical period between 17 and 20 DAH when the larva's consumption rates begin to determine whether it will be a fast-or-slow-growing larva.

Energetics of Growth Differences in Cultured Summer Flounder, *Paralichthys dentatus*: Prey Consumption and Absorption Efficiency by Newly-settled Juveniles

Tessa L. Simlick[1], Robin S. Katersky[1, 2], and David A. Bengtson[2]

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212 Rogers Avenue, Milford, CT 06460

Since fast growth is a desired quality in hatchery-raised fish, it is of interest to the aquaculturalist to study the bioenergetic causes of intraspecific differences in growth rate. We have examined the differences in consumption and absorption efficiency of fast-growing *vs* slow-growing newly-settled juvenile summer flounder, *Paralichthys dentatus*. In particular, we investigated five cohorts of juveniles derived from individual male x individual female crosses. Consumption and absorption efficiency were measured in order to determine partly the "scope for growth" for newly-settled summer flounder. Size differences appear within a cohort in the mid- to late-larval stages, such that the fastest-growing fish settle first and the slowest growing fish settle last. Upon settling, fish were graded into three tanks: grade 1 (fast-growing fish), grade 2 (medium-growing fish), and grade 3 (slow-growing fish). Food consumption rate, absorption efficiency, and specific growth rate data were collected to compare rates for: (1) fish of different sizes (15, 20, 25, and 30 mm) within a cohort, (2) fast-growing (grade 1) and slow-growing (grade 3) fish within a cohort, and (3) fish from different sets of parents. Fish were removed from the stock tank, measured by image analysis, and placed randomly into 2-L bowls (n=5 fish per bowl). The consumption rate was measured by the difference between the initial ration given to the fish and the remaining uneaten nauplii after the 24-hr feeding experiment. Absorption efficiency was determined by calculating the difference between the amount of energy ingested and the energy egested as fecal matter. Energy (in joules) was measured indirectly through a wet oxidation process. Fecal material (from each of the replicated tanks) was collected onto a preweighed glass fiber filter after a 24-hr feeding trial and subjected to a strong oxidizing agent (potassium dichromate) which was then titrated in order to determine the oxygen consumed in the experiment (= energy consumed). Food consumption rates, absorption efficiencies, and specific growth rates did not differ between fast- and slow-growing fish at 15 mm; however, significant differences did become apparent at larger sizes, although they were not continuous over the size range and among all cohorts. Food consumption rates decreased on a weight-specific basis, with increasing size, in each of the five cohorts. Significant differences between food consumption rate, absorption efficiencies and specific growth rate were observed among fish from different sets of parents.

The Oxygen Consumption, Ammonia Excretion and Scope for Growth of Newly-settled Summer Flounder, *Paralichthys dentatus*

Robin S. Katersky[1, 2], Tessa L. Simlick[1], and David A. Bengtson[1]

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[2]*National Marine Fisheries Service, Milford Laboratory*
212 Rogers Avenue, Milford CT 06460

The time of metamorphosis in summer flounder larvae from within a single cohort and between cohorts is variable due to large differences in the growth rates of individual fish. Differences in fish size leave the slower-growing fish more susceptible to cannibalism and stress. In laboratory experiments metamorphosed fish were graded into three tanks: grade 1 (fast-growing fish), grade 2 (medium-growing fish), and grade 3 (slow-growing fish). Oxygen consumption and ammonia excretion data were collected to compare rates between 1) fish of different sizes (15, 20, 25, and 30 mm), 2) fast-growing (grade 1) *vs.* slow-growing (grade 3) fish within a single cohort, and 3) cohorts with different sets of parents. The results showed a significant difference in O_2 consumption between fish of different sizes; however, there was no significant difference between fast- and slow- growing fish from within or between cohorts. The peak SDA (specific dynamic action) was ~2 times the baseline rate of O_2 consumption. There was also a significant difference in ammonia excretion for fish of different sizes and between fast- and slow-growing fish at some sizes (30 mm, cohort 2; 15 mm, cohort 3). Post-prandial ammonia excretion rates were 3.61 and 5.20 times the endogenous rates for grade 1 and grade 3 fish, respectively, and accounted for ~3% of the ingested energy. These results have been incorporated with concurrent research on the food consumption and the absorption efficiency to create an energy budget for newly-settled summer flounder using the Scope for Growth index.

Correlations among Stage-specific Abundances of Larval and Juvenile Winter Flounder near Millstone Point, CT and their Relation to Year-class Strength

Donald J. Danila[1], Ernst Lorda[1], and J. Dale Miller[1, 2]

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Monitoring studies of winter flounder have been conducted in the vicinity of Millstone Point in eastern Long Island Sound since 1976. Various indices of abundance were determined: adults spawning in the Niantic River during late winter-early spring and resulting female egg production, larvae of four developmental stages in the Niantic River and Bay from late winter through late spring, demersal age-0 juveniles in the Niantic River and Bay during summer, age-0 fish in late fall and early winter that were found throughout the study area, and age-1 juveniles present in the River and Bay concurrently with the adult spawner survey. Strongest correlations among these winter flounder abundance indices were found to occur between consecutive life stages. An exception has been a lack of correlation between a fall-winter age-0 index and succeeding age-1 indices. In fact, some persistent negative correlations have been found between age-1 abundance and several measures of adult abundance, perhaps indicating processes operating after winter flounder become age-1 that result in fewer recruits from more abundant year-classes of juveniles. In some years, events occurring during one or more of the early life history stages have led to the formation of either a relatively strong or weak year-class. Similar trends in abundance of early life history stages have been observed in some other areas of southern New England, suggesting that in many years processes occurring throughout the region likely govern the survival of winter flounder eggs, larvae or juveniles.

Distribution, Life History, and Identification of Southern U. S. *Paralichthys* with Implications for Management

Bruce A. Thompson and Andrew J. Fischer

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Although the three species of *Paralichthys* (*albigutta, lethostigma, squamilentus*) found in the northern Gulf of Mexico have been known for well over 100 years, there has been continued confusion over their specific distinctiveness and identification, and thus a lack of understanding of their life histories. The life histories of these three *Paralichthys* are "variations on a theme", with spawning offshore in the Gulf of Mexico during winter; larvae and young then move towards shore. *Paralichthys squamilentus* uses barrier island beaches as nurseries between December and May, with larger individuals migrating to deeper offshore waters for the remainder of their life. Present studies suggest this is a smaller species and does not exhibit as extreme sexual dimorphism as *P. lethostigma. Paralichthys lethostigma* primarily uses inland marshes as nurseries, with mature individuals migrating offshore to spawn, but commonly returning to estuarine waters over its life span. *Paralichthys albigutta* is now considered to be virtually absent from Louisiana waters; many museum species of young and juvenile *P. albigutta* are being misidentified *P. squamilentus*. Meristics, morphmetrics, and coloration distinguish the three species, and based on an analysis of these characters suggest that *albigutta* and *lethostigma* are closely related with s*quamilentus* sister to this pair. Management plans for these species must include different strategies for habitat protection and harvest restrictions.

Abundance, Density, and Length Frequency of Juvenile English Sole in Pacific Coast Estuaries from 1998-2000

Christopher N. Roper and Donald R. Gunderson

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Fishes with pelagic egg and larval stages are typically susceptible to dramatic fluctuations in population abundance and many have demonstrated strong correlation between physical variables and recruitment. English sole have extended pelagic egg and larval stages before settlement and movement to juvenile habitat in estuaries. The objective of this study was to examine trends in juvenile sole abundance, density, and length of frequency patterns in four nursery estuaries along the Oregon and Washington coasts. Trawl surveys were conducted in June and August of 1983-1988 and 1998-2000 in Grays Harbor, 1985-1988 and 1998-2000 in Willapa Bay and in Coos Bay and Yaquina Bay from 1998-2000. In 1998-2000 English sole abundance was lowest in Yaquina Bay and highest in Willapa Bay, and was related to the area of the estuary. In some years there were two pulses of recruitment to the estuaries shown by the bimodal length distribution indicating differences in time of settlement. Patterns in abundance, density, and length of juveniles in the estuaries may reflect inter-annual and inter-regional differences in egg and larval transport, development and growth rates due to increased water temperatures, or estuarine conditions during El Nino.

The Distribution and Abundance of Juvenile American Plaice, *Hippoglossoides platessoides*, in the Southwestern Gulf of Maine, New Hampshire

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As part of a study investigating the ecological impacts of shrimp trawling on juveniles of four species of benthic fish, two sites were sampled in the southwestern Gulf of Maine during the summer and winter of 1998 and 1999. One of the sites is within a regular shrimp-trawling ground, while the other is separated from it by a distance of 1.19 km and is designated as the "no trawl" site. Analysis of these samples indicated that of the four species sampled American plaice, *Hippoglossoides platessoides*, was predominant in the samples both spatially and temporally. It was more abundant at the site where shrimp trawling is known to occur than at the site where trawling is absent. Furthermore, *H. plattessoides* was more abundant at the two sites in summer than in the winter.

This distribution and abundance pattern was correlated with trawling activity and a number of environmental variables (sediment organic content, sediment particle-size composition, macroinvertebrate abundance, and microtopography) to determine possible relationships. Most of these variables were found to vary naturally between the two sites, masking any impact of trawling. Hence, the diet and size-composition of *H. platessoides* were also analyzed to provide further insight into any relationships between its abundance patterns and the selected variables, especially from the perspective of assessing the relative potential of the two sites as nursery and refuge habitats to juveniles of this species.

Plasticity in Size, Age and Development at Settlement of Individually Reared Winter Flounder (*Pseudopleuronectes americanus*) Larvae

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Metamorphosis in insects and amphibians is often accompanied by a niche shift, *e.g.,* an aquatic mosquito larva to a terrestrial adult. Studies examining differences in growth rates in insect and amphibian larvae suggest these taxa express a degree of plasticity in the timing of metamorphosis which allows an organism to metamorphose (*i.e*., shift niches) at an optimal size and time that will maximize its fitness. Flatfish exhibit life history traits similar to insects and amphibians; they undergo a niche shift from pelagic larvae to benthic juveniles while experiencing a change in morphology. Thus, it is conceivable that the same environmental pressures selecting for phenotypic plasticity in insects and amphibians (resource availability, predation, abiotic factors, etc.) may have the same impact on larval flatfish enabling them to quicken or delay their settlement and/or development in order to maximize their fitness. In this study we examined how developmental stages (flexion and eye migration) coincided with the timing of settlement and how these factors varied between individuals with different growth rates. Individuals were reared in 4-L flow-through tubs in a temperature-controlled (10ºC) tank, and individuals were checked daily for eye migration progress and video images were taken (nondestructively) every 2-4 days to collect sizes and flexion data. Preliminary results indicate that although differences in growth rates produced high variation in larval size at, and time of, settlement, there was less variation in stage of development at settlement. This suggests that settlement and development may be independent of somatic growth (size), but the timing of settlement is dependent on development.

Temperature Effects on Embryonic Developmental Rates, Size at Hatching and Survival of Yolk-sac Larvae of Summer Flounder, *Paralichthys dentatus*

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Summer flounder, *Paralichthys dentatus*, has a geographically broad and protracted spawning season. Because spawning occurs from waters of southern Georges Bank to North Carolina, continues through the autumn, and progresses from northerly to southerly areas, eggs are likely to experience a wide range of thermal conditions. The objective of this study was to quantify the effects of water temperature on the rate of embryonic development and the size and condition of larvae at hatching. Eggs obtained from broodstock were allocated to 1 of 10 constant temperatures (8.0 to 24.25ºC) immediately after fertilization. Eggs were sampled every 4 hours until hatching to provide accurate, temperature-dependent ages from fertilization to expression of each of 6 developmental characters that can be scored on field-collected eggs. At hatching, larvae were videotaped live and/or preserved for purposes of size and yolk content determination. A subset of larvae was maintained to evaluate the effect of temperature on resilience of yolk-sac larvae to starvation. No eggs hatched at temperatures < 10.0 ºC. Median ages to developmental events varied inversely and curvilinearly with temperature. Median ages at hatching ranged from 39 hr at 24.3 ºC to 178 hr at 11.5 ºC. Sizes at hatching were greatest at the cooler temperatures that produced viable larvae. Yolk-sac sizes at hatching varied inversely with the lengths of larvae at hatching. Survival to hatching was maximal at intermediate temperatures. The resilience of yolk-sac larvae to starvation varied inversely with temperature but was affected little by the temperature that these larvae experience as embryos. It is our intention to apply laboratory-based estimates of the temperature-dependent expression of embryonic features to eggs from ichthyoplankton collections. This application should support improved estimates of egg production and mortality rates in nature.

Metamorphosis in Summer Flounder: Manipulation of Thyroid Status to Modify Growth, Development, and Settling Behavior

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In the aquaculture of summer flounder (*Paralichthys dentatus*), high variability in growth rate (GR), development rate (DR) and settling behavior during metamorphosis leads to increased labor through the grading out of settled juveniles and to mortality through cannibalism. Our goal was to evaluate manipulation of thyroid status as a means to modify GR, DR and settling behavior of metamorphosing summer flounder. Premetamorphic flounder were treated with an exogenous thiourea (TU, 30 ppt, an inhibitor of thyroxine (T4) synthesis). Following removal of TU, metamorphosing flounder were exposed to either exogenous T4 (T4-Na salt, 100 ppb, dissolved in 1 ml of DMSO) or 1 ml of DMSO only. A control (no TU, then DMSO only, when appropriate) was used for comparison. Settling in the control was prolonged, with the > 60% (< 20% to >80%) settlement interval occurring over 9 d. TU inhibited settling over 16 days (d) of treatment. Discontinuation of TU treatment induced rapid settling after a 2-d delay, with the >60% interval reduced to 5 d. Addition of T4 further accelerated settling, resulting in a 1-d delay and reduction of the >60 % interval to 3 d. Settling rate was affected by treatment, with the TU-T4 treatment-settling rate significantly greater than the control. Additionally, both TU and TU-T4 treatments significantly increased length at settling in comparison to the control. GR and DR were calculated from length and developmental stage data collected before and after TU treatment and after T4 treatment. Eight days after completion of TU treatment, both GR and DR were significantly lower than rates in the control. In contrast, TU followed by T4 treatment resulted in GR and DR that were not significantly different than the control. Percent survival was unaffected by TU treatment. A priority for aquaculture is the reduction of both the labor associated with size grading, and the mortality due to cannibalism in the high stocking density, non-food limiting rearing environment. Our results suggest that manipulation of thyroid status to synchronize settling behavior and modify settling rates should reduce the effort associated with grading out settled juveniles. In addition, synchronization of settlement, combined with the ability to alter GR and DR, may potentially limit the exposure of pelagic larvae to cannibalistic attacks from newly-settled juveniles.

Ontogenetic Transitions During the First Year of Life in Two Cohorts of Windowpane, *Scophthalmus aquosus*

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Ontogenetic transitions in habitat, morphology, and otolith microstructure were examined in young-of-the-year (YOY) windowpane, *Scophthalmus aquosus* (Mitchill), an abundant flatfish in the Middle Atlantic Bight. The goal of this research was to identify and examine transitions that occur during early life and determine how these events might affect subsequent patterns of growth and survival of YOY windowpane. Differential habitat utilization between spring- and fall-spawned cohorts was evident, whereby spring-spawned fish were collected in estuarine, inlet, and ocean habitats while fall-spawned were collected primarily in the ocean. Densities of spring-spawned windowpane captured with planktonic gears (~ 2-20 mm standard length, SL) peaked in May in all habitats (estuary, inlet, and ocean, 10.3, 67.9 and 39.3 per 1000 m^3, respectively) and declined significantly by July. A second peak of planktonic windowpane occurred in October, when fall-spawned (~ 2-10 mm SL) reached densities of approximately 13 per 1000 m^3 in the ocean. Spring-spawned windowpane appeared in demersal gears initially in the inlet and ocean in May (~6-13 mm SL), but the first demersally-captured windowpane did not appear in the estuary until June and at ~24-32 mm SL. Settled, fall-spawned windowpane appeared in the inlet and ocean in October, with a peak in abundance occurring in the ocean in December. Various morphological characters were developed incompletely in larvae (4.0-8.0 mm SL), but most of these were well developed in juveniles (>35.0 mm SL). A transitional phase of development occurred between 8.0-27.0 mm SL, during which time 50% of the characters examined were developed incompletely. This developmental transition occurred in fish that were similar in size to field-captured windowpanes that had recently settled to demersal habitats. Examination of otolith microstructure in field-captured windowpane, belonging to both cohorts (n=31, 6-35 mm SL and n=22, 7-33 mm SL, respectively) revealed that the formation of accessory growth primordia coincides with a transitional settlement period, and the completion of eye migration (~8-20 mm SL). Back-calculated growth rate estimates for spring-spawned windowpane were significantly faster than those for fall-spawned windowpane and these differences could produce differential rates of survival for the two cohorts during the first year of life.

Gonadotropin-releasing Hormone Affects Sperm Production of Atlantic Halibut (*Hippoglossus hippoglossus*)

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Under simulated natural photoperiod, Atlantic halibut (*Hippoglossus hippoglossus)* ovulate between January and May depending on temperature and individual variability. However, spermiation commences about 1-2 months prior to female ovulation. This asynchronous production of milt and eggs frequently results in reduced sperm production and higher spermatocrit levels later in the spawning season, while some females may produce viable oocytes. Poor quality milt at this time adversely affects fertilization and, thus, production capabilities. Gonadotropin-releasing hormone (GnRH) has been used on cultured fish to stimulate or enhance spermiation. In this study, spermatocrit levels of Atlantic halibut were measured throughout the reproductive season. When spermatocrit exceeded 80%, eight males were injected with a slow-release GnRH analogue (D-Arg^6-Pro^9-NEt salmon GnRH; Ovaplant ®; 150 g; sGnRHa). Control fish were implanted with placebo implants (no sGnRHa). After two weeks half of the males that received an initial implant received another implant. After a single injection of an implant, spermatocrit levels decreased from 90% to 60% in one week. However, in fish receiving one initial implant, spermatocrit again increased to 90% within 30 d. When fish received an additional implant 2 weeks after the first, spermatocrit never exceeded 70%. Fertility of milt from males treated with sGnRHa was not significantly different from those of control fish.

Larval Winter Flounder Stock Identification Using Microelements: First-year (2000) Analysis and Preliminary Results

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Inductively-coupled mass spectrometry was utilized to obtain measurements of the quantities of a suite of 30 elements from whole winter flounder (*Pseudopleuronectes americanus*) larvae. The training set consisted of 46 second-stage larvae taken from three separate spawning areas namely, the Niantic River, Connecticut River, and the Thames River, with 13, 17, and 16 individuals, respectively. Preliminary feature selection methods reduced the number of elements substantially, and later discriminant analysis procedures provided further refinements. Classification accuracy by discriminant analysis was good with only a few (2-3) misclassifications in the entire training set. However, a neural network classifer provided even better classification results, providing a classification accuracy of about 98 percent. The neural network was then applied to 53 entrainment samples of winter flounder larvae from the Millstone Nuclear Power Station. Only two larvae were classified as being of Niantic River origin with the majority being classified as Thames River fish. We conclude the method is very promising and suggest larger samples to more accurately and precisely determine power plant effects.

Winter Flounder, *Pseudopleuronectes americanus*, Stock Enhancement in New Hampshire: Evaluating Release Locations

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The feasibility of winter flounder stock enhancement is being investigated in New Hampshire. Of particular interest is the development of optimal release strategies, including optimal release size, season, and site. To determine an optimal release site, three areas were selected in the Great Bay Estuary based on substrate type, bathymetry, and food availability.

During the summer of 1999, an experiment was conducted in which caged, cultured winter flounder were monitored at each of the three sites. Growth, survival, and temperature were measured weekly. In addition, substrate composition and food availability were determined from core samples. The results from the cage study indicate that the two sites in the upper estuary may be more optimal for released flounder than the one site at the mouth of the estuary. Although there was no difference in survival between the fish at the three sites, the fish grew significantly faster at the upper estuary sites where the water temperature was generally warmer. These sites had more prey available to the flounder and contained less gravel than the site at the mouth of the estuary.

Trawling was conducted and core samples were collected from these three sites over a two-year period. Data compiled from these efforts corroborate the enclosure experiment. These data and results from the summer pilot release will be presented as well.

The Effects of Scallop Dredging on a Recently-settled Continental Shelf Flatfish: Disturbance or Status Quo?

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Impacts of mobile bottom fishing gear on demersal fisheries have received increased attention in the scientific, trade, and popular press. However, previous findings do not necessarily extend to all environments, life stages, and/or gear types. The juvenile stage is one life stage that may be particularly sensitive to disturbance as it generally lasts longer than the larval stage and involves a complex transition from a three-dimensional, pelagic environment to a two-dimensional, benthic one. We examine the impact of scallop dredging on a recently-settled continental shelf fish in the context of a storm-dominated continental shelf system (the New York Bight). Open ocean experimental manipulations at three depths (45, 67, 88 m) using replicated control/treatment effects (BACI design) were used to investigate the immediate and longer-term impacts of a dredging event on recently-settled juvenile yellowtail flounder (*Limanda ferruginea*) and their benthic nursery habitat. Submersible surveys were used to examine temporal changes in *Limanda* abundance and habitat composition, while future work will address aspects of growth (otolith microstructure) as well as distribution of benthic infauna. Using concomitant buoy data (NOAA buoy #44025, Long Island), we also examined the physical regime of the region as it relates to critical groundfish settlement windows. Finally, natural and anthropogenic disturbances are discussed in the context of the New York Bight as a whole.

Dynamic Habitat in a Temperate Estuary and Spatio-temporal Variation in the Growth of an Early Juvenile Flatfish

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The effects of spatial and temporal habitat variation on early juvenile winter flounder (*Pseudopleuronectes americanus*, Walbaum; 10-30 mm standard length (SL)) growth was examined using field enclosure techniques in a temperate Northwest Atlantic estuary. Enclosures (N=5; 3 fish enclosure^{-1}) were deployed at each of 12 fixed stations established along gradients of temperature, salinity, and sediment organic content in the Navesink River-Sandy Hook Bay estuarine system, New Jersey. Three 12-day runs of the experiment were performed over 40 consecutive days in late May through June, 1999.

The spatial pattern of flounder growth rates (Range = 0-0.9 mm SL d^{-1} enclosure^{-1}) varied in time. Initial fish size was not a significant source of growth variation and the dynamic growth pattern was related to habitat variation. Generalized additive modeling indicated that growth was highest at relatively cool temperatures (< 22ºC), low salinities (<24ppt) and high prey densities (>0.6 individuals cm^{-2}) Analysis in trends in partial growth related to each parameter suggested that temperature and salinity gradients were responsible for growth variation at coarse spatial scales, while variation at fine spatial scales was related to prey availability and unmeasured factors (*i.e.*, residual growth). However, the relative strengths of influence of temperature and salinity on coarse-scale growth patterns changed over time. During the first run of the experiment, temperatures were cool (< 22ºC) throughout the estuary and growth was highest in the river at locations where optimal prey densities and salinities intersected within the estuary. During later runs, however, the three habitat parameters were out of phase in space as a result of spatial variation in spring warming. Average growth rates declined and the region of highest relative growth shifted downstream to the bay where temperatures were optimal but salinities and prey availability were suboptimal. Thus, the dynamic growth patterns we observed appeared to be related to the effects of multiple and nested habitat parameters that promoted rapid growth during periods when optimal conditions for the parameters intersected within estuary, but this spatial synergy of optimal conditions was transitory.

Current strategies of marine habitat conservation typically involve the preservation of areas defined by relatively stable fine-scale habitat features easily identified by human observers. Our study suggests that habitat suitability for growth of a commercially important fish is defined by a suite of spatially and temporally variable habitat parameters and as a result the location and suitability of nursery habitat can change rapidly in time.

Recruitment of Summer Flounder Larvae to Chesapeake Bay: Larval Flux at an Inlet

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Ichthyoplankton samples were collected continuously from the mouth of Chesapeake Bay for 56 hours during mid November 1999. Nets were fished near the surface, middle and bottom of the water column and changed hourly. Tidal velocity and direction were monitored continuously using an Acoustic Doppler Current Profiler (ADCP) and temperature, salinity, and depth were sampled with a CTD every half hour. Summer flounder larvae were removed from samples, counted, measured, and staged. Temperature and salinity plots indicated three water masses during sampling. During strong northwest winds at the beginning of sampling, warm water was blown out of the bay on an ebb-tide dominated cycle. This was followed by a cold-water mass during an ebb and flood tidal cycle. The final 40 hours consisted of several tidal cycles of higher salinity water. Summer flounder larvae were collected over the entire sampling interval and were more abundant in the higher salinity water mass. Stages of larvae ranged from pre-eye migration and late flexion (stages E and F) to complete eye migration and post flexion (stage I). Larvae apparently were higher in the water column during flood tides, but other patterns are being evaluated. Concentration of larvae and current velocity were combined to calculate larval flux. Early-stage larvae spend more time in the water column during ebb tides; their upchannel flux is lower than later stage larvae.

Use of a Rhode Island Salt Pond by Juvenile Winter Flounder, *Pseudopleuronectes americanus*

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We used a 1.75-m^2 drop-ring sampler in June and July of 2000 to quantify populations of juvenile flatfishes and other small nekton in Ninigret Pond, Rhode Island. The drop sampler was deployed in approximately 1 m of water from a boom mounted on the bow of a small boat. Abundance of juvenile winter flounder (15-95 mm) was 3.9 ± 0.8 (SE) inds/m^2 in seagrass habitat. Abundance was somewhat higher in non-vegetated habitat, though direct comparisons between habitats are difficult because of possible gear bias.

This non-vegetated habitat consisted of either macroalgae or bare sand/mud. Our results point to the importance of all these habitat types as valuable nurseries for flatfishes in Ninigret Pond. We suggest that similar habitats in other coastal areas may likewise be very important. We also report on high abundances of other fishes, crabs and shrimps in both seagrass and non-vegetated/macroalgal habitats in Ninigret Pond.

Experimental Evaluation of Ontogenetic Diet Transitions in Summer Flounder, *Paralichthys dentatus*, Using Stable Isotopes as Diet Tracers

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Summer flounder, *Paralichthys dentatus*, is a rapidly growing flatfish that increases in length by nearly 100-fold during its first year of life. A series of diet shifts including transitions in trophic status (*e.g.*, planktivory to piscivory) as well as in diet source (*e.g.*, habitat shifts) occurs during this period. The diet source and trophic status of a consumer can be deduced from the proportion of stable isotopes of both carbon and nitrogen. The rate at which the isotopic signal from a new diet is reflected in the tissue of a consumer (*i.e.*, isotopic turnover) is a function of both growth and metabolic rate. It is therefore likely that temperature and ontogenetic state affect turnover rate. Our experiments were designed to determine the effect of temperature and ontogenetic state on the rate of isotopic turnover for summer flounder. We examined three ontogenetic diet transitions: 1) endogenous (yolk) nutrition to exogenous (zooplankton) feeding, 2) zooplankton type 1 (rotifers) to type 2 (*Artemia*) and 3) zooplanktivory to piscivory. Each of these experiments was conducted at two temperatures (13 and 18ºC) using two related families of summer flounder as replicates. First feeding was monitored by destructively sampling larvae at 8 time-points from hatching to 16 days after first feeding. The transition between two types of zooplankton was initiated by moving 40 rotifer-fed summer flounder larvae (6-7 mm TL) into a rearing container where only *Artemia* nauplii were offered as food. These *Artemia*-fed larvae were sampled destructively at 9 time-points from 0 to 32 days after the change in diet. The zooplanktivory to piscivory transition started with individually contained summer flounder (37.4, 3.9 mm TL, 0.61, 0.15 g wet weight) that were offered 3 to 6 juvenile winter flounder per day and destructively sampled from 0 to 32 days after the change in diet. Summer flounder in each of the three experiments displayed a clear shift in isotopic signals. Turnover was slower at cooler temperatures. First feeding resulted in the most rapid turnover rate and piscivory the slowest. The relative contribution of growth and cellular rebuilding to isotopic turnover as well as the observed shift in isotopic signal during a trophic shift will be discussed.

Restriction Endonuclease Characters of Flatfish Ribosomal DNA May Help Resolve their Phylogeny

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Pleuronectiformes comprise a specialized assemblage of flatfishes which first appeared about 50 million years ago. There are about 570 flatfish species, representing 123 genera grouped into 11 families. One of the synapomorphic characters of the pleuronectiform fishes is the bilateral asymmetry of their eyes in juveniles and adults. Their current phylogeny based on morphology is in question due to homoplasy and convergence of many of these characters. However, molecular diversity of their genes may provide additional information to aid in resolving their phylogeny. The present study characterizes the rDNA of two flounders, summer flounder (*Paralichthys dentatus*) and winter flounder (*Pseudopleuronectes americanus*), and a sole, hogchoker (*Trinectes maculatus*) using restriction endonucleases (RE).

Nuclear DNA was extracted from each species and was digested with *Bam*HI, *Eco*RI, *Hind*III, *Pst*I and *Pvu*II individually and in combinations. The DNA fragments were separated by agarose gel electrophoresis, transferred to a nylon membrane following the Southern transfer technique and hybridized with digoxigenin-labeled *Xenopus laevis* rDNA (pXlr101A). The hydrids were immunoreacted with anti-digoxigenin alkaline phosphates conjugate, that was detected using Lumi-Phos 350 and recorded on Kodak XAR X-ray film. Sizes of the rDNA RE fragments were determined graphically in order to construct a RE map for each species. Using parsimony analysis, the rDNA data alone supported winter flounder to be closer to summer flounder than hogchoker, while 23 of their well accepted apomorphic morphological characters could not resolve this relationship.

An Examination of Winter Flounder (*Pseudopleuronectes americanus*) Larvae Genetic Stock Structure in Long Island Sound

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Winter flounder larvae (*Pseudopleuronectes americanus*) populations isolated from adjacent geographical areas in Long Island Sound (LIS) and larvae entrained by the Millstone Nuclear Power Plant were examined for genetic differences with microsatellite markers (from Dr. Susan Douglas, Institute for Marine Biosciences, Halifax, Nova Scotia). Larvae (stages 1 and 2) were isolated in March 2000 from the Niantic River (NR) stations (A & B at 3 m of depth), the Thames River (TR, due west of the Submarine Base at 12-m depth) and from Plum Bank (PB, about 2.5 miles west of the river mouth, at the bottom) with 202- and 333-µm mesh nets. Four hundred twenty-three larvae were collected from these reference populations (135, 135, and 444, respectively). Three hundred sixty larvae (stage 2 through 4) entrained at the power plant were collected during April, May and June 2000. Genomic DNA was isolated from each larva and microsatellite markers were amplified in a standard PCR reaction mixture with one of three primer sets (hHiD34a & b or hHiI29a & b or hHiJ24a & b). Microsatellite products were analyzed by high-resolution PAGE (polyacrylamide gel electrophoresis). Microsatellite product sizes were determined by comparison to size markers. Primer hHiD34a & b gave 5 products between 200-325 bp, primer hHiI29 a & b gave 9 products between 80-225 bp and primer hHiJ24a & b gave 19 products between 100-188 bp.

The initial analysis examined the genetic differences between the 3 source populations by calculation of F_{ST} and genetic differences. The 2 most separate geographical populations, PB and TR, had a very significant genetic differentiation ($F_{ST} = 0.436$). There was also a large genetic differentiation between the NR and TR populations ($F_{ST} = 0.0384$). The NR and PB populations were more genetically related ($F_{ST} = 0.0158$). Entrained larvae were then compared to each reference population to determine their most likely source geographical location. Data were analyzed by SPAM (Software Program for Analyzing Genetic Mixtures). During the month of April, 3% of the entrained larvae were from the NR, 55% from the TR, 39% from PB, and 3% from an unknown area. In May, less than 1% of the entrained larvae were from the NR or PB areas and 98% were from the TR. In June, 10% of the larvae were from the NR and PB areas, 78% from the TR, and 1% from an unknown area. These results are in agreement with the identification of entrained larva source locations by elemental analysis (data not shown).

The Role of Carbonic Anhydrase in Renal Sulfate Secretion by Winter Flounder (*Pseudopleuronectes americanus*)*

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Winter flounder plasma sulfate is stable at about 0.6 mM and is regulated by the kidneys through tubular secretion. This process is vital for excretion of the large inorganic sulfate load gained from ingestion of seawater. Transport studies on isolated flounder renal tubule basolateral (BLM) and brush-border membrane (BBM) vesicles revealed that sulfate entry, interstitium-to-cell, across the BLM is in exchange for OH^-, whereas the exit, cell-to-lumen, at the BBM is in exchange for HCO_3^-. Carbonic anhydrase (CA) apparently enhances this process through dehydroxylation of HCO_3^- (*Am. J. Physiol*. 276: F288-F294, 1999). Teleosts that tolerate varying salinities obviously have the capability to regulate renal excretion. Cortisol has been implicated in the adaptation of teleosts to seawater; indeed, it was termed the "seawater adapting" hormone (*Am Zool*. 15:937-948, 1975). Renfro (*Am. J. Physiol*. 257: R511-R516, 1988*)* showed that adaptation of seawater winter flounder to 10% seawater (SO_4-free) resulted in sulfate clearance ratios less than one and decreased BBM HCO_3^- SO_4^{2-} exchange. Clearance ratios greater than one and enhanced BBM HCO_3^- SO_4^{2-} exchange in these animals were restored by daily injections of the long-lived cortisol analogue, dexamethasone (60 μ g/100 g bd. wt.), for 5 days. This profound effect of glucocorticoid on renal sulfate secretion, together with the recent observation that renal sulfate secretion was CA dependent, prompted examination of the influence of cortisol on Ca-dependent renal sulfate secretion. Flounder renal epithelial cells were isolated as previously described (*Am. J. Physiol.* 251: F424-F432, 1986) and plated to confluence on native rat tail collagen. Unidirectional $^{35}SO_4^{2-}$ fluxes were determined for 14-day old cultures mounted in Ussing chambers. Net secretion rates were calculated from unidirectional fluxes. Control tissues had complete tissue culture medium with 5 g/ml hydrocortisone (cortisol). Removal of cortisol 5 days prior to flux determination caused a significant 20% reduction in net secretion. Methazolamide (100 μM), a specific CA inhibitor, reduced net secretion to about 43% in both control and no-added-cortisol tissues. The methazolamide-insensitive component of flux was unaffected by cortisol removal. These treatments had no significant effect on the transepithelial electrical properties of the tissues. We conclude that cortisol controls the methazolamide-sensitive component of renal sulfate secretion possibly through renal CA functional activity.

*Supported by NSF-IBN9604070, NINDS-NS10475, and NSF-IBN9808616

The Effects of Hypoxia on Growth and Hematology of Juvenile Summer and Winter Flounder

Kevin L. Stierhoff and Timothy E. Targett

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The objectives of this research were to determine the effects of static and diel-cycling hypoxia on growth rate and hematocrit (HCT) of young-of-the-year summer flounder (*Paralichthys dentatus*) and winter flounder (*Pseudopleuronectes americanus*). Fish (40-80 mm TL) of both species were exposed to four static levels of hypoxia (7.0, 5.0, 3.5, and either 2.0 or 2.5 mg/l O_2) and one diel-cycling treatment (7.0-2.5 mg/l O_2). Experiments were conducted at two temperatures (20 and 25°C) in 25 ppt salinity seawater. Fish were fed *ad libitum* on live spionid polychaetes (*Marenzellaria virens*). Fish (N = 9 per treatment) were measured (0.1 mm) and weighed (± 1.0 mg) on days 0, 7, and 14 of each experiment. Daily specific growth rate (SGR =% body weight/d) was determined separately for consecutive one-week periods. Blood for HCT determinations was taken from a subsample of fish from each treatment condition on days 0 and 7 (N=5 each) and from all fish in the growth rate experiment on day 14 (N =9 per treatment).

Summer flounder at 20°C showed no significant decrease in SGR between 7.0 and 5.0 mg/l O_2. However, SGR decreased by 45% between 5.0 mg/l O_2 (SGR = 9.08% bw/d) and 2.0 mg/l O_2 (SGR = 4.9% bw/d). There appeared to be no detrimental effect of diel-cycling oxygen levels on SGR in summer flounder at 20°C.

Winter flounder at 20°C also showed no significant decrease in SGR between 7.0 and 5.0 mg/l O_2. However, SGR decreased by 65% between 5.0 mg/l O_2 (SGR = 4.35% bw/d) and 2.5 mg/l O_2 (SGR= 1.46% bw/d). Diel-cycling oxygen levels resulted in SGR values intermediate between those at 7.0 and 2.5 mg/l O_2 in winter flounder at 20°C. No increase in SGR was apparent in week 2, compared to week 1, suggesting no acclimation response in growth rate over this time scale.

Although there was a similar decrease in absolute SGR with decreasing oxygen levels in summer and winter flounder, the proportional decrease was much greater for winter flounder, due to their slower growth rate. Juvenile summer flounder appear to be less impacted by diel-cycling oxygen levels than are juvenile winter flounder. Potential HCT differences across oxygen level treatments, and between weeks 1 and 2, will be discussed for both species.

Foraging in Juvenile Summer and Southern Flounder: Effects of Light, Turbidity and Prey Type

Ursala A. Howson[1, 2] and Timothy E. Targett[2]

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The ranges of two paralichthid flounders, summer flounder (*Paralichthys dentatus*) and southern flounder (*Paralichthys lethostigma*), overlap in the South Atlantic Bight from North Carolina to Florida. Juvenile summer flounder typically feed in euryhaline habitat within the estuary and are exposed to fluctuating levels of turbidity. Juvenile southern flounder inhabit oligohaline regions of the upper estuary and forage in high-turbidity habitat. Previous field studies have indicated that juvenile summer flounder feed primarily on benthic invertebrates, while southern flounder consume epibenthic/pelagic prey. It is unclear whether these differences in prey consumption are the result of differences in foraging modes between the two species, artifacts of prey density or location, or differences in visual capabilities in turbid estuarine habitats.

Turbidity impacts foraging by decreasing reactive distance between predator and prey and may thus impact growth and survival of estuarine fishes. Particulates in the water column decrease light levels by absorbing light and reduce visibility by scattering light. To separate the effects of absorption (reduction in light intensity) with those of scattering (reduction in visibility), foraging rates of summer and southern flounder were determined in clear water (1 NTU) at five light levels (2×10^{14} to 6×10^{11} quanta sec^{-1} cm^{-2}) and in darkness. Foraging rates were also compared across four turbidity levels (1, 11, 20 and 40 NTU) at 6×10^{12} quanta sec^{-1} cm^{-2}. The effects of light intensity and turbidity on prey preference were also examined by conducting separate trials with two different prey types (mysid shrimp as epibenthic/pelagic prey, polychaetes as benthic prey). To examine the effects of turbidity on locomotory behavior, activity of summer and southern flounder (food withheld for > 12 hr) was assessed at 1, 10, 20, and 40 NTU.

An Experimental Analysis of Size-specific Predator-prey Interactions between Juveniles of Summer Flounder, *Paralichthys dentatus*, and Winter Flounder, *Pseudopleuronectes americanus*

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The outcome of an encounter between a predator and its prey often depends on the sizes of the interacting individuals. This size dependency is more likely to be expressed in species that grow through a wide range of body sizes. Knowledge of the growth rates of the potentially interacting species, as well as the timing of their spatial and temporal overlap, is especially important for life periods during which rapid growth is exhibited. During their early juvenile period, summer flounder (*Paralichthys dentatus*) and winter flounder (*Pseudopleuronectes americanus*) are a good candidate pair for the analysis of such size- and growth-based predator-prey interactions. Juveniles of these two flatfishes overlap spatially in inshore waters from Southern New England through New Jersey. The ingress and settlement of summer flounder typically precedes the settlement of winter flounder. Summer flounder become piscivorous at small sizes (25 mm TL) and grow rapidly as spring temperatures increase. In this experimental study we used recently-settled juvenile summer and winter flounder in order to evaluate 1) the size combinations at which winter flounder were at risk to summer flounder, 2) the magnitude of risk as a function of relative body sizes of the two species, and 3) the attack rates, satiation levels, and the handling times of summer flounder when encountering winter flounder that fall within the range of vulnerable sizes. Regarding critical size combinations of the two species, we show that the boundary separating the region resulting in predation from that in which winter flounder is invulnerable to predation is linear, is tightly related to summer flounder mouth gape, and is accurately represented by a proportionality constant, *i.e.*, winter flounder is at risk to predation if summer flounder TL is > 2.2 winter flounder TL. We also show that the function relating the number of winter flounder consumed versus the number offered is a decelerating ('Type II') functional response with a calculated attack rate and handling time of 0.5 and 1.2 hr, respectively, which vary with the specific size combination of prey and predator. Lastly, we provide estimates of temperature-dependent growth for juveniles of both species and suggest how these parameters could drive the outcome of predator-prey interactions at the population level.

Response of YOY Winter Flounder to Sediment Biogeochemicals

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The location of juvenile winter flounder (*Pseudopleuronectes americanus*) habitat at the estuarine sediment-water interface subjects these fish to an environment characterized by precipitous biogeochemical gradients. Changes in habitat quality variables (dissolved oxygen, sulfide, nitrite, ammonium, etc.) of macrobiotic behavioral significance can occur on scales as small as millimeters and hours. Even in high energy areas of the Hudson-Raritan Estuary with coarse sandy substrates, oxygen disappears in the upper millimeters of the sediment, and water column oxygen concentrations have been observed to decrease from 250 µM to less than 30 µM over a few days. Bedja *et al*. (1992) have shown that such periodic reductions of oxygen concentrations (to 60 µM) reduce growth rates of young-of-the-year (YOY) winter flounder. To simulate exposure to biogeochemicals at the seabed, wild-caught YOY winter flounder were held in a sand-bottom, vertical-flow tank in which half the sediment area was perfused with manipulated seawater. Temperature was maintained between 19.4 to 21.5ºC over the course of 26 experiments and temporal biogeochemical gradients and fish locations were recorded. The winter flounder responded to declining O_2 at approximately 100 µM (3.2 mg/L) by moving to more oxygenated water. Sulfide treatment produced a more complex response, apparently requiring concentrations in excess of 15-20 µM sulfide and a steep rate of increase for initiation of movements. In exploratory trials with nitrite (50 µM) and ammonium (100 µM) we found no clear response though the fish appeared to be in distress.

Field and Laboratory Observations on Feeding Behavior of Newly-settled Winter Flounder, *Pseudopleuronectes americanus*

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Field and laboratory investigations were conducted to examine feeding by newly-settled winter flounder, *Pseudopleuronectes americanus*, on two co-occurring calanoid copepods, *Eurytemora affinis* and *Acartia hudsonica*, in the Navesink River estuary, NJ. During the spring, these prey are present when winter flounder initiate their demersal lifestyle. Epibenthic zooplankton were collected concurrently with winter flounder in May 1998 and 1999. Although both calanoid species were in the estuary during the two-year survey, *E. affinis* was consumed nearly to the exclusion of *A. hudsonica* by newly settled winter flounder. Annually, *E. affinis* and *A. hudsonica* had similar size distributions in field collections, indicating that species choice was not size selective. However, when preying on *E. affinis,* winter flounder preferred the larger-sized organisms. In single-species laboratory experiments, *E. affinis* and *A. hudsonica* were consumed equally by newly-settled winter flounder (19-23 mm TL), but there were more strikes made toward *E. affinis.* Despite the lower catch efficiency, *E. affinis* was selected over *A. hudsonica* when the prey species were offered together in equal numbers. The selection for *E. affinis* over *A. hudsonica* by newly-settled winter flounder may be the result of behavioral or morphological differences in the prey species.

GIS Mapping of Winter Flounder (*Pseudopleuronectes americanus*) Data for Rhode Island Waters, an Effort to Identify Essential Fish Habitat (EFH)

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Geographical Information Systems (GIS) can be used to optimize sampling, explain the spatial distribution of coastal fish populations, identify Essential Fish Habitat (EFH), and to assist decision makers in ecological impact assessment of non-point source pollution, oil spills, and natural disturbances of fisheries resources. The objective of this project was to develop the first geo-spatial databases and coverages of sampling stations and winter flounder populations from data collected by the Rhode Island Department of Environmental Management, Division of Fish and Wildlife during 1979 through 1999, in Narragansett Bay, Rhode Island Sound, and Block Island Sound. The resulting geo-spatial coverages will serve as the basis for further spatio-temporal analysis of winter flounder spawning and nursery habitat, adult habitat, migratory routes, and help define Essential Fish Habitat (EFH) for this species in Rhode Island waters. This effort was a pilot study to evaluate the feasibility of using this methodology for future assessment of other important finfish and shellfish species. Future studies will integrate marine habitat and land use information for a more comprehensive ecological assessment of Rhode Island fisheries.

Abstracts
Poster Presentations

Comparison Between Two Methodologies for Batch-marking Adult Winter Flounder: Preliminary Results

Donald J. Danila

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Spawning adult winter flounder have been collected in the Niantic River since 1977 as part of long-term monitoring studies for assessment of impact of Millstone Nuclear Power Station. Before release, healthy fish larger than 15 cm (1977-82) or 20 cm (1983 and thereafter) were freeze-branded in a specific location with a number or letter made by a brass brand cooled in liquid nitrogen. Marks and brand location were varied in a manner such that the year of marking was apparent for recaptured fish. These mark-recapture data are used with the Jolly-Seber model to estimate population size. Throughout most of the study, some fish were captured having an apparent freeze brand, but the specific mark was indistinguishable. Thus, some information was being lost. Since this study began, more technologically advanced techniques have been developed to batch-mark fish. In both 1999 and 2000, a second mark was applied to freeze-branded winter flounder using fluorescent-pigmented particles injected into the dorsal fin with New West Technologies Biometrix System 1000 Micro-JectTM or SuperMicro-JectTM portable injectors. Color and fin position of the photonic mark were unique by year. The efficacy of this marking system versus freeze branding was examined by noting the presence or absence of each mark following the recapture of these fish within the year or one year later for 1999 fish. Difficulties were encountered in marking fish in 1999 using the less powerful Micro-JectTM injector, but these were overcome in 2000 by using a stronger spring in this injector. Nevertheless, larger (ca. >35 cm) flounder were more difficult to mark than smaller fish with both injectors. Preliminary results indicated that nearly all recaptured fish retained the freeze brand (at least up to 1 year). Although most of the fish recaptured within a few weeks of marking had a photonic mark, only a fraction of them had retained fluorescent pigment after one year. Results may have been influenced to some degree by an inability to use UV light in the field to examine for photonic marks, despite constructing a special viewing box for that purpose.

A Family of Pleurocidin-like Antimicrobial Peptides from Winter Flounder

Jeffery W. Gallant and Susan E. Douglas

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Low molecular weight antimicrobial peptides are an important component of the innate immune system in animals, yet they have not been examined widely in fish. We report genomic sequences encoding pleurocidin-like antimicrobial peptides from the winter flounder, *Pseudopleuronectes americanus* (Walbaum), as well as reverse transcription-PCR products from skin and intestine-two mucosal surfaces that form the first defensive barrier to microcrobes. Alignment of the predicted polypeptide sequences shows a conserved hydrophobic signal peptide of 22 amino acids followed by approximately 25 amino acids that are able to form an amphipathic a-helix, followed by a conserved acidic portion. Southern hybridization analysis indicates that related peptides are encoded in the genomes of other flatfish species. Northern and RT-PCR analyses of RNA show that two of the pleurocidin genes are expressed predominantly in the skin whereas two other genes are expressed mainly in the intestine. RT-PCR assays of total RNA from winter flounder larvae of different ages indicate that the pleurocidin gene is first expressed at 13 days post-hatch and provide the first evidence of developmental expression of antimicrobial peptides in fish.

Spatial Distribution of Flounder Collected in Channel and Shoal Habitats of the New York and New Jersey Harbor Estuary as Related to Sediment Characteristics

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The New York and New Jersey Harbor estuary provides spawning, nursery and foraging habitats for several commercially and recreationally important flatfish species. As part of a study related to deepening Harbor navigation channels, shoal and channel habitats in the New York and New Jersey Harbor estuary were sampled during a year-long monitoring program. One objective of the sampling program was to determine the spatial and seasonal distribution of flatfish in the Harbor. Fish were collected using a 9-m bottom trawl at twenty stations ranging in water depth from 9 to 51 ft (MLW). Species distribution and relative abundance was compared to bottom-sediment characteristics at each sampling station. Bottom-sediment varied from hard sand to smooth sand, with some stations exhibiting a combination of sediment types.

Winter flounder (*Pseudopleuronectes americanus*), smallmouth flounder (*Etropus microstomus*), windowpane flounder (*Scophthalmus aquosus*), fourspot flounder (*Paralichthys oblongus*), and summer flounder (*Paralichthys dentatus*) were collected at both shoal and channel stations; however, their spatial distribution varied between stations and among Harbor sampling areas. Species distribution varied with bottom-sediment characteristics. Winter flounder dominated most catches, but was predominant over smooth mud habitat. Windowpane flounder was the dominant species collected over hard sand. Stations with mixed sediment characteristics provided the greatest variety of species. Additional information on bottom sediment characteristics and flatfish distribution would aid in determining habitat preference in estuarine areas.

Increase in Numbers of Smallmouth Flounder, *Etropus microstomus*, in the Ichthyoplankton of Narragansett Bay and Mount Hope Bay, Rhode Island

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The smallmouth flounder is a small flatfish found in nearshore waters and estuaries ranging from Cape Cod to Cape Hatteras. The center of distribution appears to be the Chesapeake Bight where they are one of the most numerous flatfish species collected in the icthyoplankton. The eggs and larvae are fairly common off southern New England and Cape Cod but are rare in Narragansett and Mount Hope Bay. Impingement data from the Braden Point Power Plant in Mount Hope Bay, Massachusetts shows an increasing upward trend in smallmouth flounder numbers since 1985. Previous ichthyoplankton surveys in Narragansett Bay collected few smallmouth flounder, but a recent survey begun in June 2000 collected relatively large numbers of eggs. Sampling conducted in 1972-1973 showed no smallmouth flounder, but the eggs were not described until 1980. To date, in the 2000 ichthyoplankton collection, the eggs comprise 6% of the ichthyoplankton and are ranked 4th in abundance. Water temperatures have been rising in Narragansett and Mount Hope Bays and it will be interesting to see if this increase in the smallmouth flounder will be a permanent long-term trend.

GIS Mapping of Winter Flounder (*Pseudopleuronectes americanus*) Data for Rhode Island Waters, an Effort to Identify Essential Fish Habitat (EFH)

Wilfrid Rodriguez[1], Peter August[1], and J. Christopher Powell[2]

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Geographical Information systems (GIS) can be used to optimize sampling, explain the spatial distribution of coastal fish populations, identify Essential fish Habitat (EFH), and to assist decision makers in ecological impact assessment of non-point source pollution, oil spills, and natural disturbances of fisheries resources. The objective of this project was to develop the first geo-spatial databases and coverages of sampling stations and winter flounder populations from data collected by the Rhode Island Department of Environmental Management, Division of Fish and Wildlife during 1979 through 1999, in Narragansett Bay, Rhode Island Sound, and Block Island Sound. The resulting geo-spatial coverages will serve as the basis for further spatio-temporal analysis of winter flounder spawning and nursery habitat, adult habitat, migratory routes, and help define Essential Fish Habitat (EFH) for this species in Rhode Island waters. This effort was a pilot study to evaluate the feasibility of using this methodology for future assessments of other important finfish and shellfish species. Future studies will integrate marine habitat and land use information for a more comprehensive ecological assessment of Rhode Island fisheries.

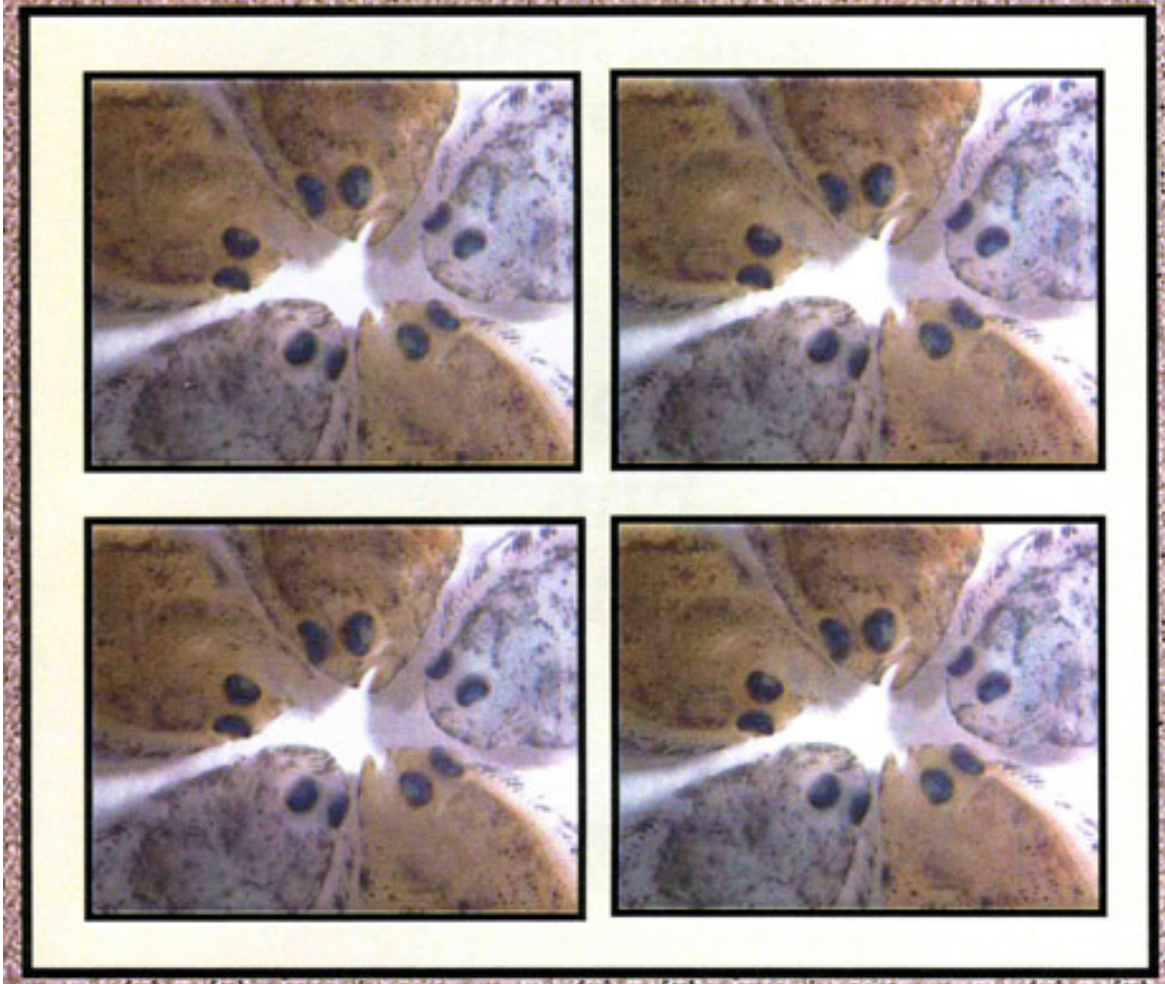
Density-dependent Changes in Area of Habitat Occupied by Georges Bank Yellowtail Flounder (*Limanda ferruginea*)

Travis Shepherd and Matthew K. Litvak

University of New Brunswick
Saint John, New Brunswick, Canada E2L 4L5

We examined density-dependent changes in habitat-usage patterns through the relationship between abundance and the area of habitat occupied by George's Bank yellowtail flounder (*Limanda ferruginea*). Canadian Georges Bank spring groundfish survey data from the Department of Fisheries and Oceans (1986-2000) were used to estimate density and the area of habitat occupied. Annual densities were estimated on a fixed-square grid (0.1º longitude x 0.1º latitude) by interpolating (kriging) between known survey values. Annual global densities were estimated from the mean of the grid estimates. Area of occupied habitat was estimated by contouring the grid estimates to a minimum patch density threshold (the minimum yellowtail density which defines occupancy) and calculating the area within that contour. A range of values for minimum patch density was used (*e.g.*, 1 fish, 5 fish, 10 fish, etc.) in order to examine density-dependent aspects in the rate of change of occupied habitat area. Increases in area of occupied habitat were related closely to increases in global density. The nature of this relationship was dependent on minimum patch density; *i.e.*, exponential for low patch density thresholds and asymptotic for high thresholds. These results have implications for the spatial definition of the Georges Bank stock, transboundary management issues, and the possibility for systematic changes in catchability.

Flatfish Biology Conference



December 10-11, 2002
Westbrook, Connecticut

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Flatfish Biology Conference
December 10-11, 2002, Westbrook, Connecticut

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Eighth in a series of Flatfish Biology Conferences

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Flatfish Biology Conference

December 10-11, 2002, Water's Edge Resort, Westbrook, Connecticut

Oral Presentations

Tuesday, December 10th

8:00 a.m. **Registration/Coffee, Continental Breakfast**

8:45 a.m. Welcome and Introduction
Renee Mercaldo-Allen
Anthony Calabrese
National Marine Fisheries Service
Northeast Fisheries Science Center
Milford, CT

John Boreman, Director
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, MA

Session I

Penny Howell, Chair
Connecticut Department of Environmental Protection
Fisheries Division, Old Lyme, CT

9:00 a.m. All-*trans* Retinoic Acid Stimulates Pigment Development in Summer Flounder
Michael Baron
University of New Hampshire, Department of Zoology, Institute for the Development and Evolution of Wet Animals, Durham, NH

9:20 a.m. Spatial and Temporal Patterns of Hogchoker (*Trinectes maculatus*) Reproduction in the Delaware Bay Estuary
Christina M. Hodgson, Stephen G. Piotrowski, Kenneth W. Able, and Thomas M. Grothues
Rutgers University Marine Field Station, Tuckerton, NJ

9:40 a.m. Global Patterns of Species Richness for Flatfishes (Order *Pleuronectiformes*)
Thomas A. Munroe
National Marine Fisheries Service, Northeast Fisheries Science Center, National Systematics Laboratory, Smithsonian Institution, Washington, D.C.

10:00 a.m. Stock Structure of Yellowtail Flounder off the Northeastern United States
Steve Cadrin
National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole Laboratory, Woods Hole, MA

10:20 a.m. **Break/Coffee/Refreshments**

Session II

Donald J. Danila, Chair
Dominion Nuclear Connecticut, Inc., Millstone Environmental Laboratory
Waterford, CT

10:50 a.m. Examining the Decline of Narragansett Bay Winter Flounder
Allison DeLong and Jeremy Collie
University of Rhode Island, Graduate School of Oceanography, Narragansett, RI

11:10 a.m. Optimal Release Strategies for Winter Flounder Stock Enhancement
Elizabeth A. Fairchild and W. Huntting Howell
University of New Hampshire, Department of Zoology, Durham, NH

11:30 a.m. Metamorphosis in Summer Flounder: Manipulation of Rearing Salinity to Synchronize Settling Behavior, Growth and Development
Steven Gavlik and Jennifer L. Specker
University of Rhode Island, Graduate School of Oceanography, Narragansett, RI

11:50 a.m. **Hosted Buffet Lunch**

Session III

Ambrose Jearld, Chair
National Marine Fisheries Service
Woods Hole, MA

1:10 p.m. Active Sulfate Secretion by the Intestine of Winter Flounder, *Pseudopleuronectes americanus*
Ryan M. Pelis[1] and J. Larry Renfro[2]
[1]Mount Desert Island Biological Laboratory, Salisbury Cove, ME, and [2]University of Connecticut, Department of Physiology and Neurobiology, Storrs, CT

1:30 p.m. The Trophic Ecology of Northwest Atlantic Flatfishes: A Case Study of George's Bank
Jason S. Link[1], Michael J. Fogarty[1], Karen Bolles[2], Cheryl G. Milliken[3], and Rich W. Langton[4]
[1]National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole Laboratory, Woods Hole, MA, [2]Marine Research Institute, Reykjavik, Iceland, [3]Massachusetts Division of Marine Fisheries, Pocasset, MA, [4]Buccoo Reef Trust, Scarborough, Tobago

1:50 p.m. Tidal Rhythms in Winter Flounder
Beth Phelan
National Marine Fisheries Service, Northeast Fisheries Science Center, James J. Howard Marine Sciences Laboratory, Highlands, NJ

2:10 p.m. Winter Flounder Mortality on an Estuarine Nursery Ground:
A Preliminary Analysis of Coarse and Fine-scale Habitat Patterns
John P. Manderson[1], Jeff Pessutti[1], Patricia Shaheen[1], and Francis Juanes[2]
[1]National Marine Fisheries Service, Northeast Fisheries Science Center, James J. Howard Marine Sciences Laboratory, Highlands, NJ and [2]University of Massachusetts, Department of Natural Resources Conservation, Amherst, MA

2:30 p.m. **Refreshment Break**

Session IV

Mark Dixon, Chair
National Marine Fisheries Service
Milford, CT

3:00 p.m. Use of Video to Assess Juvenile Winter Flounder Densities and Habitats
Lisa Meng[1], Giancarlo Cicchetti[1], and Steve Raciti[2]
[1]U.S. Environmental Protection Agency, Atlantic Ecology Division, Narragansett, RI and [2]Vassar College, Poughkeepsie, NY

3:20 p.m. The Genetic Stock Structure of Larval and Juvenile Winter Flounder (*Pseudopleuronectes americanus*) in Connecticut Waters of Eastern Long Island Sound and Estimations of Larval Entrainment
Joseph F. Crivello[1], Donald J. Danila[2], Ernesto Lorda[2], Milan Keser[2], and Edward F. Roseman[2]
[1]University of Connecticut, Departments of Physiology and Neurobiology and Marine Sciences, Storrs, CT and [2]Dominion Nuclear Connecticut, Inc., Millstone Environmental Laboratory, Waterford, CT

3:40 p.m. Formation of Year-class Strength of the Niantic River, CT Winter Flounder Stock-When and Where Variation Occurs and Suggestions on How and Why
Donald J. Danila and Edward F. Roseman
Dominion Nuclear Connecticut, Inc., Millstone Environmental Laboratory, Waterford, CT

4:00 p.m. **Poster Set-up**

5:00 p.m. **Hosted Mixer and Poster Session**

Wednesday, December 11

8:15 a.m. **Registration/Coffee/Continental Breakfast**

Session V

Jay Burnett, Chair
National Marine Fisheries Service
Woods Hole, MA

9:00 a.m. The Smallmouth Flounder, *Etropus microstomus*, in Narragansett Bay: Looks Like They are Here to Stay
Grace Klein-MacPhee[1], Aimee Keller[1], Dennis Erkan[2], and Michael Scherer[3]
[1]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI, [2]Rhode Island Department of Environmental Management, Division of Fish and Wildlife, Jamestown, RI, and [3]Marine Research Inc., Falmouth, MA

9:20 a.m. Review of the Ecology of Winter Flounder in Narragansett and Mt. Hope Bays: Why the Decline?
Rodney A. Rountree, Brian Rothschild, Lou Goodman, Wendall Brown, Yalin Fan and Liuzhi Zhao
University of Massachusetts at Dartmouth, School for Marine Science and Technology, New Bedford, MA

9:40 a.m. Sand Shrimp, *Crangon septemspinosa,* Predation on Juvenile Winter Flounder: Effect of Temperature on Predator Functional Response and Foraging Behavior
David L. Taylor
University of Rhode Island, Graduate School of Oceanography, Narragansett, RI

10:00 a.m. Can the Predator Pit Hypothesis Explain the Retarded Recovery of Southern New England Winter Flounder Stocks?
Saul B. Saila[1] and Ernesto Lorda[2]
[1]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI, and [2]Dominion Nuclear Connecticut Inc., Millstone Environmental Laboratory, Waterford, CT

10:20 a.m. **Break/Coffee/Refreshments**

Session VI

Chris Powell, Chair
Rhode Island Division of Environmental Management
Jamestown, RI

10:40 a.m. Winter Flounder Essential Fish Habitat (EFH) Conservation Recommendations
Michael Ludwig
National Marine Fisheries Service, Habitat Conservation Division, Milford, CT

11:00 a.m. Genetic Stock Identification and Mass-balance Modeling Determine Contribution of Niantic River Winter Flounder Larvae to Power Plant Entrainment
Edward F. Roseman[1], Donald J. Danila[1], Ernesto Lorda[1], and Joseph Crivello[2]
[1]Dominion Nuclear Connecticut, Inc., Millstone Environmental Laboratory, Waterford, CT and [2]University of Connecticut, Departments of Physiology and Neurobiology and Marine Sciences, Storrs, CT

11:20 a.m. The Enigmatic Deep-water Witch Flounder of the Mid-Atlantic Bight
Jay Burnett and Susan Wigley
National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole Laboratory, Woods Hole, MA

11:40 a.m. Culture of Atlantic Halibut (*Hippoglossus hippoglossus*) in Offshore Net Pens
W. Huntting Howell, Michael Chambers, and Nathan Rennels
University of New Hampshire, Department of Zoology, Durham, NH

12:00 p.m. **Hosted Buffet Lunch**

1:00 p.m. **Adjourn Meeting**

Poster Session

Tuesday, December 10, 5:00 p.m.

Squamation, Pigmentation and Asymmetry in Summer Flounder
David M. Boynton and Jessica A. Bolker
University of New Hampshire, Department of Zoology, Institute for the Development and Evolution of Wet Animals, Durham, NH

The California Halibut as an Aquaculture Candidate: A Comparison to Other Flatfish
Douglas E. Conklin[1], Raul H. Piedrahita[2], Jean-Benoit Muguet[1], German E. Merino[2, 3], and Margarita Cervantes-Trujano[1, 2]
[1]University of California, Department of Animal Science, Davis, CA, [2]University of California, Department of Biological and Agricultural Engineering, Davis, CA, and [3]Universidad Catolica del Norte, Departamento de Acuicultura, Coquimbo, Chile

Initial Evidence of Vertical Migration of Winter Flounder (*Pseudopleuronectes americanus*) in a New Jersey USA Estuary
Mary Carla Curran[1], Robert J. Chant[2], Kenneth W. Able[3], and Scott M. Glenn[2]
[1]Savannah State University, Marine Science Program, Savannah, GA, [2]Rutgers University, Institute of Marine and Coastal Studies, New Brunswick, NJ, and [3]Rutgers University, Marine Field Station, Institute of Marine and Coastal Studies, Tuckerton, NJ

The Contribution of Southern Flounder and Summer Flounder to the Recreational Fishery of the Southeastern USA
Mary Carla Curran and Donna E. McDowell
Savannah State University, Marine Science Program, Savannah, GA

Mortality of YOY Winter Flounder Held on Newark Bay Sediment: Some Unplanned Observations
Andrew F. J. Draxler and Kristina M. Salvati
National Marine Fisheries Service, Northeast Fisheries Science Center, James J. Howard Marine Sciences Laboratory, Highlands, NJ

Abundance, Distribution, and Condition of Hogchokers (*Trinectes maculatus*) in the Hudson River Estuary April-September, 2002
Ivan Ferron and Chris Chambers
National Marine Fisheries Service, Northeast Fisheries Science Center, James J. Howard Marine Sciences Laboratory, Highlands, NJ

Temporal Changes in Behavior of the Blackcheek Tonguefish, *Symphurus plagiusa*
Jody L. Frost and Mary Carla Curran
Savannah State University, Marine Science Program, Savannah, GA

Site Fidelity Patterns of Sub-legal Summer Flounder in Virginia Waters from Angler-assisted Tagging Program Data, 2000-2002
Jon A. Lucy[1] and Claude M. Bain, III[2]
[1]Virginia Sea Grant Marine Advisory Program, Virginia Institute of Marine Science, College of William and Mary, Gloucester Pt., VA, and [2]Virginia Saltwater Fishing Tournament, Virginia Marine Resources Commission, Virginia Beach, VA

Embryonic and Larval Staging of Summer Flounder, *Paralichthys dentatus*
Gabriela M. Martinez and Jessica A. Bolker
University of New Hampshire, Department of Zoology, Institute for the Development and Evolution of Wet Animals, Durham, NH

Seasonal Changes in Blood Chemistry of the Yellowtail Flounder, *Limanda ferruginea*
Renee Mercaldo-Allen, Margaret A. Dawson, Diane Kapareiko, and Catherine A. Kuropat
National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT

Winter Flounder Stock Enhancement
Ben Morgan[1] and Michael Scherer[2]
[1]Llennoco, Inc., Chatham, MA, and [2]Marine Research Inc., Falmouth, MA

Winter Flounder (*Pseudopleuronectes americanus*) Spawning Areas in New Haven Harbor
Jose J. Pereira, Ronald Goldberg, and Paul Clark
National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT

The Feeding Behavior of Atlantic Halibut, *Hippoglossus hippoglossus*
Gwynne Schnaittacher and David Berlinsky
University of New Hampshire, Department of Zoology, Durham NH

Histological and Biochemical Comparison of Skin Development in Normal and Malpigmented Summer Flounder
Amy Van Cise, Tanya F. Hakala, and Jessica A. Bolker
University of New Hampshire, Department of Zoology, Institute for the Development and Evolution of Wet Animals, Durham, NH

Skeletal Anomalies in Offshore Species of Flatfish, American Plaice: A Comparison with Winter Flounder
John Ziskowski
National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT

Abstracts
Oral Presentations

All-*trans* Retinoic Acid Stimulates Pigmentation Development in Summer Flounder*

Michael Baron

University of New Hampshire, Department of Zoology
Institute for the Development and Evolution of Wet Animals
Durham, NH 03824

Dietary carotenoids and Vitamin A are thought to be important factors in the development of flounder pigmentation. However, supplementation of these compounds has yielded mixed results (Kanazawa, 1993, Takeuchi *et al.,* 1995). Retinoic acid (RA) is a hormone derived from carotinoids and Vitamin A. RA has been used to stimulate pigmentation development in the Japanese flounder, *Paralichthys olivaceus* (Miwa and Yamano, 1999, Haga *et al.,* 2002). This study examined the effect of RA on pigmentation development in summer flounder, *Paralichthys dentatus*. Larvae were immersed in 5 and 10 nM concentrations of RA for a period of 10 days preceding metamorphosis. Fish were reared for an additional 20 days following treatment. Nearly all fish in the study developed normal ocular side pigmentation. Fish in the control group also expressed normal development on the blind side. However, over 60% of the fish in the treatment groups expressed partial or total pigmentation of the blind side. These results support a role for retinoic acid in the pigmentation development of flounder.

*Supported by New Hampshire Sea Grant #111381 to Jessica Bolker.

Spatial and Temporal Patterns of Hogchoker (*Trinectes maculatus*) Reproduction in the Delaware Bay Estuary

Christina M. Hodgson, Stephen G. Piotrowski, Kenneth W. Able, and Thomas M. Grothues

Rutgers University, Marine Field Station
Tuckerton, NJ 08087

Hogchokers (*Trinectes maculates*) are one of the dominant fishes in Delaware Bay based on a seven-year (1996-2002) survey of intertidal and subtidal creeks. In order to learn more about their basic life history, specimens were collected during May to November from 1999-2002 to determine spatial and temporal patterns of reproduction. Seven marsh creek systems representing lower (12-18 ppt), middle (5-7 ppt) and upper (0-5 ppt) bay along the New Jersey side of Delaware Bay were sampled monthly with replicate tows of a 4.9-meter otter trawl (6-mm cod end mesh). Initial observations based on length frequency distributions and spatial/temporal patterns show potentially mature individuals (85-140-mm TL) frequenting the lower bay during spawning season where salinity is highest. Intermediate sizes (60-85 mm TL) were found further upstream while abundance of young-of-year (YOY) (35-60 mm TL) peaks were found where salinity is the lowest. The differentiation of size classes along a salinity gradient is consistent with the Dovel (1969) model. Verification by calculating a monthly Gonadosomatic Index (GSI) over three years helps define the reproductive pattern of hogchokers in response to seasonal shifts of environmental factors such as temperature and salinity.

Global Patterns of Species Richness for Flatfishes (Order Pleuronectiformes)

Thomas A. Munroe

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Northeast Fisheries Science Center
National Systematics Laboratory
Smithsonian Institution
Washington, DC 20560

Comparisons of species richness estimates reveal interesting patterns regarding the ecological biogeography of flatfishes. Within all regions, flatfishes are most diverse where extensive continental shelves with complex habitats are located in shallow water. Generally, diversity of marine flatfishes increases along continental shelves from polar to equatorial waters, with maximum diversity of flatfish assemblages usually occurring on tropical and subtropical continental shelves within each ocean. Flatfish diversity is also high at continental shelf areas where components from different faunal provinces intermix. Lower than expected diversity is noted on continental shelf regions where cold-water currents and upwelling occur. In all oceans, but especially in the West Pacific, flatfish diversity is lower at insular compared with continental locations at the same latitude. Within each geographic region, continental islands (usually larger with heterogeneous soft-sediment habitats) generally support higher numbers of flatfish species than do oceanic islands (usually smaller with less habitat complexity) located in the same region. Among four tropical marine regions, the highest diversity of flatfishes occurs in the western Pacific (125 species compared with 45 in the western Atlantic, 43 in the eastern Pacific and only 38 species in the eastern Atlantic). Diversity of flatfishes in northern temperate and boreal regions is greater than that of corresponding areas in the southern hemisphere. Far fewer species are known from temperate and boreal areas in the Atlantic and eastern Pacific oceans compared with those from the western Pacific at comparable latitudes.

Stock Structure of Yellowtail Flounder off the Northeastern United States

Steve Cadrin

National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole Laboratory
Woods Hole, MA 02649

This interdisciplinary study evaluated spatiotemporal patterns of abundance, geographic variation in growth and maturity, larval transport, morphometry, and genetics of the yellowtail flounder in the northeastern United States. The results suggest that yellowtail flounder found on the principal U.S. fishing grounds should be managed as separate stocks, despite genetic homogeneity. Two "harvest stocks" of yellowtail flounder have significantly different patterns of abundance and biomass over time, with a boundary from southwest Georges Bank to Nantucket. Geographic patterns of size and proportion mature at age indicate two "phenotypic stocks" of yellowtail flounder, with a boundary along the northern edge of Georges Bank to Nantucket. Therefore, yellowtail from the southern New England fishing grounds form a separate harvest stock than those on the Georges Bank or Cape Cod grounds, and Cape Cod yellowtail are a separate phenotypic stock than those on Georges Bank or off southern New England. No significant differences were detected between southern New England and Mid-Atlantic yellowtail, or between those from the Cape Cod grounds and the Gulf of Maine. Inferred larval movement reveals a passive southwest drift along the continental shelf. Morphometric analysis showed sexual dimorphism and significant difference between yellowtail from U.S. waters and those sampled off Newfoundland, but little morphometric variation among U.S. areas. Genetic analysis also found little variation among U.S. samples. U.S. yellowtail flounder resources appear to comprise a single genetic stock, but significant variation in critical life history attributes and different patterns of abundance over time suggest that three fishery management units exist: southern New England-Mid Atlantic, Georges Bank, and Cape Cod-Gulf of Maine.

Examining the Decline of Narragansett Bay Winter Flounder

Allison Delong and Jeremy Collie

University of Rhode Island
Graduate School of Oceanography
Narragansett, RI 02882

The Narragansett Bay winter flounder population has experienced a severe decline in abundance over the last two decades, as evidenced by catches in the Rhode Island Department of Environmental Management, Division of Fish and Wildlife and University of Rhode Island, Graduate School of Oceanography standardized trawl surveys. Although regional populations (southern Massachusetts, Long Island Sound, Rhode Island Sound) of winter flounder have also experienced declines, they do not appear to have been as severe as those observed in Narragansett Bay and several of these other populations have begun to recover under strict fishing regulations. The principle objective of this study was to use field data to describe the decline of winter flounder in Narragansett Bay and to find evidence of those factors that have led to the decline and have kept it from rebuilding at the rates experienced by regional populations. We first present a comparative analysis of several regional populations: Narragansett Bay, Mt. Hope Bay, southern Massachusetts, Long Island Sound and Niantic River, CT. To do this, we estimated the abundance of and mortality rates between 7 life stages: egg, larval, young-of-the-year (YOY) spring, YOY fall, age-1 spring, age-1 fall, and age-2 spring. We then examined environmental variables that may have affected winter flounder abundance and mortality rates within Narragansett Bay. The variables considered included age-class abundance, year, water temperature, precipitation, fishing mortality, seal abundance, double-crested cormorant abundance, chlorine discharge from wastewater treatment facilities, dissolved oxygen, salinity and power plant flow and heat load. Stepwise regression and regression tree analyses were performed to determine those environmental variables that best explain changes in stage-specific mortality rates.

Optimal Release Strategies for Winter Flounder Stock Enhancement

Elizabeth A. Fairchild and W. Huntting Howell

University of New Hampshire
Department of Zoology
Durham, NH 03824

As part of a program to assess the feasibility of winter flounder, *Pseudopleuronectes americanus*, stock enhancement, optimal release size, site, season, and condition of caged cultured juvenile winter flounder were evaluated.

To determine optimal release size, the predator-prey size relationship between winter flounder and the green crab, *Carcinus maenas*, was examined. The number of flounder killed per day was significantly higher (31%) in winter flounder < 20-mm compared to all other larger fish size classes (4-8% killed/day). Additionally, these fish were attacked at a faster rate than any other fish size class. These results suggest that only flounder > 20-mm should be released.

Field studies were conducted in three potential release sites in the Great Bay Estuary during 1999-2001 to determine optimal release site and season. Optimal site selection was based on growth and survival of caged cultured fish in relation to water temperature, prey availability, and sediment composition. Optimal season was selected based on the temporal distribution, abundance, and sizes of wild flounder, and their primary prey and predators. Within the estuary, Broad Cove was chosen as the optimal release site due to high fish growth rates coupled with the high prey availability and sandy substrate.

Although predators were equally abundant throughout the summer months, early summer was determined as the most appropriate time for winter flounder releases because prey were most abundant and wild flounder sizes were similar to the optimal release size for cultured fish. The condition of the cultured flounder was studied through a series of experiments to evaluate their vulnerability to predation based on behavior, color, and substrate preference. Cultured winter flounder reacted differently than wild flounder when exposed to cues from a potential predator and were significantly more vulnerable to predation by birds, regardless of fish color. Additionally, cultured flounder selected sediments consisting of small grains and of colors matching their own pigment.

Prior to any winter flounder enhancement effort, pilot-scale releases should be conducted to test release strategies.

Metamorphosis in Summer Flounder: Manipulation of Rearing Salinity to Synchronize Settling Behavior, Growth and Development*

Steven Gavlik and Jennifer L. Specker

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Narragansett, RI 02882

In the aquaculture of summer flounder (*Paralichthys dentatus*), the inherent variation in growth and settling behavior during metamorphosis may lead to cannibalism and necessitate increased labor due to size grading. Our goal was to use an environmental salinity change to synchronize settling behavior and produce a uniformly sized cohort of juvenile summer flounder. Early metamorphic summer flounder (Age: 41 dah) were exposed to either a 5-day fluctuating (30-20-30-20-30 ppt; "Flux") or a single (30-20 ppt; "Low Salinity") drop in rearing salinity. The Flux group was reared at 30 ppt while the Low Salinity group remained at 20 ppt until sampling at 61 dah. A control (continuous 30 ppt) was used for comparison. For all treatments, the initial n=60 fish/tank, and 3 tanks/treatment. Settling behavior in the control was prolonged, with the Peak Settlement Interval (PSI; defined as the interval beginning on the day the first 20% settled until the day 80% had settled) requiring 8 days. Settling behavior was synchronized by the Low Salinity treatment, with the PSI reduced to 5 days. The Flux treatment negatively affected settling behavior with the PSI increased to 10 days. By 61 dah, average fish size was increased by the Low Salinity treatment (19.3 ± 0.5 mm), but not the Flux treatment (17.2 ± 0.4 mm), compared to the control (17.6 ± 0.5 mm). Developmental stage at 61 dah was significantly increased in the Low Salinity treatment (3.2 ± 0.1) in comparison to the Flux (2.9 ± 0.1), but not the control (3.1 ± 0.1). However, the Low Salinity treatment reduced variance in development. To confirm the positive effects of the Low Salinity treatment, a second experiment was performed. A single salinity drop (30-20 ppt) at 37 dah (Low Salinity2) was compared to a control (continuous 30 ppt). In this experiment, the Low Salinity2 treatment did not synchronize settling. The PSI for the Low Salinity2 treatment was 14 days while the control was 13 days. Additionally, by 58 dah, average fish length (16.8 ± 0.2 mm) and developmental stage (2.9 ± 0.1) in the Low Salinity2 treatment was not significantly different than the control (16.1 ±0.2 mm; 2.8 ± 0.1). Variance in both length and developmental stage at 58 dah in the Low Salinity2 treatment was not significantly reduced compared to the control. Percent survival was unaffected by treatment in both experiments. In aquaculture, the effective synchronization of settling behavior and growth through environmental manipulations may reduce the labor costs associated with size grading. A fluctuating salinity regime is not effective in this regard. A single drop in rearing salinity may result in synchronization of settlement and development and an increase in size. Future work will consider combining a single salinity drop (to 20 ppt) treatment with our previously reported thyroid hormone manipulation treatment (Gavlik *et al*., 2002).

*This work was supported by Rhode Island Sea Grant, under NOAA Grant No NA16RG1057.

Active Sulfate Secretion by the Intestine of Winter Flounder, *Pseudopleuronectes americanus**

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Marine teleosts are hypoosmotic to their surrounding environment and must continuously drink seawater to avoid dehydration. It is widely accepted that water uptake across the marine teleost intestine is driven by active absorption of monovalent ions (Na^+ and Cl^-). However, intestinal transport of divalent ions ($SO_4$2-, Mg^{2+}, and Ca^{2+}) has been less intensively studied. In this study, $SO_4$2- transport by winter flounder (*Pseuodopleuronectes americanus*) intestine was characterized in Ussing chambers. Under short-circuited conditions and 1 mM $SO_4$2- on both sides, net active $SO_4$2- secretion (blood-to-lumen) occurred (7.42 ± 0.63 nmoles x cm^{-2} x hr^{-1}). Treatment with NaCN (10 mM) or ouabain (0.1 mM) inhibited net secretion indicating dependence on metabolism and the plasma membrane Na^+ gradient. Luminal treatment with the anion exchange inhibitor 4, 4'-diisothiocyanatostilbene-2-2'-disulfonic acid (DIDS, 0.2 mM) also inhibited net secretion. Removal of Cl^- alone, and Cl^- and HCO_3- together from the luminal bath solution reduced net SO42- secretion. Removal of HCO_3- alone stimulated net secretion. Sulfate uptake into foregut brush-border membrane vesicles was stimulated by a trans-Cl^- gradient (in>out), and unaffected by a trans-HCO_3- gradient (in>out). Short-circuiting with K^+, in=out, and valinomycin had no effect on Cl^--stimulated $SO_4$2- uptake suggesting electroneutral exchange. These data indicate that the winter flounder intestine actively secretes SO42- by exchanging for luminal Cl^-. This process may function in water absorption and in the maintenance of plasma $SO_4$2- homeostasis.

*Supported by NSF.

The Trophic Ecology of Northwest Atlantic Flatfishes: A Case Study of Georges Bank

Jason S. Link[1], Michael J. Fogarty[1], Karen Bolles[2], Cheryl G. Milliken[3], and Rich W. Langton[4]

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The Georges Bank fish community has undergone drastic changes over the past several decades, including dramatic declines in the abundance of key flatfish species. Many of these declines have been attributed to intensive fishing pressure. Flatfish are both directly susceptible to fishing gears and indirectly susceptible to the impact of those gears on the ocean bottom. Thus, Georges Bank flatfishes play multiple roles as populations highlight the need to understand the role of these fish as predators, competitors, and prey. We present estimates of relative abundance for major flatfishes from research surveys, which demonstrate that even with a recent recovering trend for a few species due to area closures, most flatfishes are far from their levels of historical abundance. Because flatfishes serve as a major energy pathway for conversion of benthic production into a form suitable for consumption by higher predators and humans, we then examined the diet of nine major flatfishes from this ecosystem including primarily polychaete- crustacean feeding or piscivorous species, with one echinoderm specialist. From this information, we assess the magnitude of predation by flatfish on other components of the food web. Additionally, we evaluate the degree of competition among the flatfishes and between the flatfishes and other fish species via diet overlap, spatial overlap, interaction coefficient estimates, and cross-correlation of population abundances. Skates are the primary competitor of flatfishes in this ecosystem and have the potential to competitively depress some flatfish populations. Finally, we explore the implications of flatfish trophic ecology with respect to different flatfish population recoveries and how the Georges Bank ecosystem may function in the future.

Tidal Rhythms in Winter Flounder

Beth Phelan

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Knowledge of endogenous rhythms provides important life history information that helps us understand the dynamic nature of habitat selection. Activity rhythms of winter flounder under controlled condition in the laboratory were observed and compared with the distribution pattern of winter flounder at high and low tides in the field. In the laboratory, all sizes of winter flounder tested (20-69 mm TL) exhibited high activity levels following high tide. Winter flounder in the field were more abundant across certain depths that shifted with the tide. It is hypothesized that winter flounder movement patterns allow them to take advantage of feeding opportunities and to avoid predation.

Winter Flounder Mortality on an Estuarine Nursery Ground: A Preliminary Analysis of Coarse and Fine Scale Habitat Patterns

John P. Manderson[1], Jeff Pessutti[1], Patricia Shaheen[1], and Francis Juanes[2]

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In the Navesink River/Sandy Hook Bay estuarine system, New Jersey winter flounder larvae are known to settle throughout the estuary but spatial settlement patterns are rapidly modified by postsettlement mortality and/or emigration. In this study, we performed chronological tethering experiments and trammel net surveys at both coarse and fine spatial scales to test the hypothesis that predation mortality was responsible for the alteration of the settlement pattern.

Coarse scale experiments showed that survivorship for tethered flounder (20-40 mm standard length) during the settlement period (late April - mid May) was variable but generally high throughout the estuary. However, as the season progressed, survival declined in the Navesink River and was lower in the region than in Sandy Hook Bay. Patterns of predator abundance and diets from trammel net collections suggest that summer flounder, searobins, and blue crabs were probably responsible for flounder mortality.

Fine scale experiments performed in the Navesink River during the post-settlement period (June -July) to examine habitat effects showed that the survivorship of juveniles (40-60 mm SL) was significantly higher in shallow water (<50 cm) than in adjacent deep habitats (>150 cm) that lacked structural complexity and were less than 30 m apart. Summer flounder appeared to be important predators in the deeper water river habitats.

Our preliminary analyses of coarse scale patterns suggest that predation pressure on juvenile winter flounder may be higher upstream in the river during the post-settlement period. However, analysis of fine scale habitat differences show that shallow water habitats including those that lack structural complexity may serve as critical refugia reducing predator encounter rates in geographic regions where predators and prey overlap.

Use of Video to Assess Juvenile Winter Flounder Densities and Habitats

Lesa Meng[1], Giancarlo Cicchetti[1], and Steve Raciti[2]

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[2]Vassar College, Poughkeepsie, NY 12604

We used a digital video camera mounted to a 1-m beam trawl together with an attached continuous recording YSI sonde and a GPS unit to quantify juvenile winter flounder densities and fish habitat in Narragansett Bay, RI. The YSI sonde measured temperature, salinity, dissolved oxygen, depth, turbidity, and chlorophyll a. We hypothesized that human-induced habitat alteration would correlate with a decrease in juvenile winter flounder densities. We sampled true-random points derived from digitization of the entire shoreline of the West Passage and the Providence River. At each random point, the camera/beam trawl/YSI unit was deployed at the water's edge, then towed out perpendicular to shore for 50-100 m, depending on the amount of macroalgae present. We sampled 80 transects from June-July 2002 and captured 603 fish representing 23 species. Winter flounder made up 60% of the catch, followed by grubby at 22%. Contrary to our expectations, juvenile winter flounder densities were greater at sites with more anthropogenic influence. Densities were highest at the head of the bay, near the city of Providence and in other semi-enclosed areas with high chlorophyll a values. When random locations were broken down into eight habitat types (beach, marsh, cobble beach, rip-rap, rock, industrial, marina and macroalgae), densities were highest near rip-rap, industrial areas, and marinas. These areas tended to be near the head of the bay or near harbors with high levels of nutrients and chlorophyll a.

The Genetic Stock Structure of Larval and Juvenile Winter Flounder (*Pseudopleuronectes americanus*) in Connecticut Waters of Eastern Long Island Sound and Estimations of Larval Entrainment

Joseph F. Crivello[1], Donald J. Danila[2], Ernesto Lorda[2], Milan Keser[2], and Edward F. Roseman[2]

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The winter flounder (*Pseudopleuronectes americanus*) is one of a number of coastal American flatfish that face intense fishery pressure and thus has been the focus of management efforts. This species has experienced dramatic declines in abundance over the past three decades with concomitant decreases in commercial and recreational fishing landings. The genetic stock structure of winter flounder larvae in Long Island Sound has not been previously characterized. Stage 1 (yolk-sac) and 2 (pre-flexion) larvae were collected from several locations in Long Island Sound known to be nursery areas for winter flounder in the spring of 2001. The genetic variations among larvae were characterized through the use of 6 microsatellite loci that had been previously reported to be highly polymorphic and heterozygous in winter flounder. The gene frequency differences were used to characterize population structure. Substantial genetic differences were seen among the putative source populations. These genetic differences appeared to be geographically based and provide evidence of genetically distinct spawning populations that appear to be temporally stable. These differences were used to characterize the most likely sources of winter flounder larvae entrained at the Millstone Power Station as well as settled juvenile winter flounder collected in the Niantic River. Samples were classified to the most likely geographical source population through use of a neural net learning algorithm. A validation of these classification results was conducted using a re-sampling scheme based on a bootstrap methodology that led to estimations of the 95% and 99% confidence intervals. These results are discussed in the context of winter flounder management issues.

Formation of Year-class Strength of the Niantic River, CT Winter Flounder Stock -When and Where Variation Occurs and Suggestions on How and Why

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Formation of year-class strength in most of the marine fishes largely occurs during the larval and early juvenile stages, yet these phases of life history are often the least understood. Long-term ecological studies of winter flounder conducted at Millstone Power Station in Waterford, CT since the early 1980s have focused on the nearby Niantic River spawning stock and have afforded the means for examining changes in abundance at sequential developmental stages during early life history. Variable annual rates of mortality in early life history can result in large differences in abundance and, hence, in year-class strength at time of recruitment to the spawning stock. Changes in survival and abundance can often vary annually, indicating that complex processes affect recruitment and that these processes may also be different from year to year. From our studies, annual abundance data are available on the adult spawning stock and female egg production during the winter-spring spawning season, larvae of four developmental stages in the Niantic River and Bay during spring, settled age-0 juveniles in the river (and for 5 years in the bay) in summer, and older (ages-0 and 1) juveniles that disperse throughout our study area in fall and winter. We describe these data and note where critical changes in abundance and mortality have occurred during certain periods of early life history. Although our sampling has been relatively consistent for about two decades, allowing good annual comparisons to be made, we have only limited information on processes likely affecting mortality and abundance. Thus, mechanisms that affect survival and recruitment are largely limited to inferences made using results from studies completed elsewhere. However, our considerable information on winter flounder in conjunction with other data gathered during our studies and elsewhere enables us to suggest potential processes that affect the formation of winter flounder year-class strength in southeastern Connecticut.

The Smallmouth Flounder, *Etropus microstomus*, in Narragansett Bay: Looks Like They are Here to Stay

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The smallmouth flounder is a small flatfish found in near shore waters and estuaries ranging from Cape Cod to Cape Hatteras. The center of distribution appears to be the Chesapeake Bight, where they are one of the most numerous flatfish species collected in the ichthyoplankton. The eggs and larvae are fairly common off southern New England and Cape Cod, but were rare in Narragansett and Mount Hope Bay. Impingement data from the Brayton Point Power Plant in Mount Hope Bay, Massachusetts shows an increasing upward trend in smallmouth flounder numbers since 1985. Previous ichthyoplankton surveys in Narragansett Bay collected few smallmouth flounder, but a recent survey begun in June 2000 collected relatively large numbers of eggs and larvae. Sampling conducted in 1972-1973 showed no smallmouth flounder eggs and few larvae, but the eggs were not described until 1980. In 1990, no eggs and few larvae were collected. In the 2000 ichthoplankton collection, the eggs comprise 16.5% of the ichthyoplankton and were ranked second in abundance, and the larvae comprised 11%, also second in abundance over the summer and fall seasons. In 2001 and 2002, the smallmouth flounder continues to be a presence, the temporal occurrence is May-October and it is present at all stations in Narragansett Bay.

Review of the Ecology of Winter Flounder in Narragansett and Mt. Hope Bays: Why the Decline?

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Winter flounder abundances have experienced dramatic declines throughout the greater Narragansett Bay estuarine system, including within Mt. Hope Bay and the Sakonnet River. In Mt. Hope Bay in particular, the decline of winter founder has been suggested to be more severe than in other regions and is often attributed to the impact of the Bryton Point Power Plant. The major reason for this assertion is that the decline in Mt. Hope Bay was coincident with a large increase in the power plants cooling water intake and effluent in 1984. We have examined time trends in the abundance of winter flounder and other species from 11 different areas within the Narragansett Bay system based on trawl data collected by the Rhode Island Department of Environmental Protection from 1979-2001. Most areas exhibit decline patterns similar to that of Mt. Hope Bay. Mt. Hope Bay declines are not significantly greater than other comparable areas, and in fact are less than declines in Greenwich Bay and the Sakonnet River. A further examination of the spatial-temporal patterns in the greater Narragansett Bay reveal that shallow semi-enclosed areas have exhibited the greatest declines and that declines are stronger for juvenile size classes than for adults. These patterns suggest an overall shrinkage of winter flounder's distribution in the bay. We also examined spatial temporal patterns in the fish assemblage and find that the Narragansett Bay fish community has undergone a dramatic shift from benthic to pelagic species. This pattern is strongest in the shallow embayments (Greenwich Bay, Sakonnet River, Mt. Hope Bay, Wickford Harbor and upper Narragansett Bay), and weakest in the deep central bay and adjacent Rhode Island Sound areas. These patterns are consistent with community structure changes associated with eutrophication. We suggest that changes in the abundance of winter flounder and fish species assemblages in Mt. Hope Bay and greater Narragansett Bay over the last 25 years is symptomatic of widespread eutrophication.

Sand Shrimp, *Crangon septemspinosa*, Predation on Juvenile Winter Flounder: Effect of Temperature on Predator Functional Response and Foraging Behavior

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Predator-prey dynamics between the sand shrimp, *Crangon septemspinosa,* and juvenile winter flounder were examined in laboratory experiments to assess the joint effects of varying prey density and temperature on shrimp foraging behavior and mortality of flounder. The functional response of shrimp to six densities of flounder was determined at two temperatures (10 and 16 °C). Moreover, the behavioral mechanisms underlying the shrimp's functional response (encounters, attacks, captures, and handling time) were quantified with visual observations and compared to the foraging parameters predicted by continuous-time functional response models. Shrimp consumption rates increased significantly with increasing flounder density, irrespective of water temperature. At low flounder densities, however, significantly more flounder were consumed at 16 °C then at 10 °C. Analysis of proportional mortality of flounder across prey density revealed a positively density-dependent (sigmoidal) type-III functional response at 10 °C, and an inversely density-dependent (hyperbolic) type-II functional response at 16 °C. Based on model parameter estimates and visual observations of predator foraging behavior, differences in functional responses were attributed to increased shrimp activity (and encounters) at higher flounder densities and temperature. These findings indicate that shrimp are capable of driving young-of-the-year flounder populations to local extinction during warm water conditions.

Can the Predator Pit Hypothesis Explain the Retarded Recovery of Southern New England Winter Flounder Stocks?

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A plausible regulatory mechanism, based on predation of early life history stages of the winter flounder, *Pseudopleuronectes americanus*, was developed utilizing a reproduction curve with three equilibrium points. This type of reproduction curve was first suggested by Walters (1986), but apparently has not been tested with empirical data to date. We fitted a five-parameter curve to the relationship between spawning stock size and recruitment to Age 1 which contained three equilibria and which explained more than 80 percent of the variability in the data from the Niantic River, Connecticut. This type of relation can arise when a predator follows a sigmoid functional response in their consumption rates of early life history stages (larvae). If the predator population remains fixed over time, the prey stock may display a stable equilibrium at two stock sizes (S_l and S_h). The stock size between these two stock sizes (S_u) is an unstable level from which the stock will collapse to a lower value or grow towards the high equilibrium value (S_h). Following the initial work of Taylor (this conference), the sand shrimp, *Crangon septemspinosus*, is implicated as a major predator of winter flounder at early life history stages. Using a segmented regression technique, we demonstrate that there is some coherence between the decline of the winter flounder stocks and the relative abundance of predators. We conclude that this work suggests that the predator pit region of the reproduction curve may account for the delayed recovery of southern New England winter flounder stocks.

Reference: Walters, C. 1986. Adoptive Management of Renewable Resources. Macmillan, New York.

Winter Flounder Essential Fish Habitat (EFH) Conservation Recommendations

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Throughout history, statutory laws have been enacted that unexpectedly create conflicts between government agency objectives. Over the last half-century, the conflicts between mandates that favor water resource development and environmental protection of those same waters have become evermore contentious. The focusing of legal intent to the point where they can occur, now, within a single agency has facilitated these mission conflicts. The passage of supplemental legislation in furtherance of either mandate has narrowed the focus and sharpened the conflict with the result that agencies are compelled to act against each other. The conflicts between the three resource agencies (US Fish & Wildlife Service [FWS], the National Oceanic and Atmospheric Administration / National Marine Fisheries Service [NMFS], the US Environmental Protection Agency [EPA]) and the US Army Corps of Engineers (Corps) are legendary. Today, as we move into the twenty-first century, the conflict between improving Port infrastructure by providing adequate access and the need to protect public trust resources living within the same waters has become a national concern. The conflict is embodied in time-of-year restrictions on dredging and disposal of sediment. Resolution of the matter is problematic because the objectives cannot be reconciled in mutual mandates or economic frameworks. For example, invocation of a seasonal window to protect aquatic resources may preclude a single, continuous dredging of a desired access channel. Valuation of the dredging and cost delays is possible, but valuing aquatic resource impacts is not an equally well-grounded practice.

Because winter flounder are unique in their spawning and early life stage characteristics, they tend to engender agency conflict and special note in EFH Conservation Recommendations. Through a series of serendipitous and focused investigations NMFS has come to be the source of much of the controversy. Species sensitivity, spawning concentration identification, and early life stage movements are the issues that have come to be the basis for negotiating resource protective objectives under the EFH program. Local knowledge of these matters is vital as are the advances made in dredging and our understanding of the impacts associated with it. Much of this interplay has taken place in the waters off the New England coastline. Today, NMFS routinely invokes seasonal constraints on any activities that might diminish winter flounder EFH from January to June.

Genetic Stock Identification and Mass-balance Modeling Determine Contribution of Niantic River Winter Flounder Larvae to Power Plant Entrainment

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Catch-per-unit-effort of winter flounder in assessment trawl surveys conducted in the Niantic River, CT has declined since the early 1980's. While the causes of this decline are speculative, the magnitude of the impact of entrainment by Millstone Power Station (MPS) on the Niantic River stock depends upon how many larvae originated from the Niantic River. Hydrodynamic modeling and tidal current drogue studies conducted in the 1970s and 1990s showed that much of the water entering MPS comes from Long Island Sound. Other winter flounder stocks are known to spawn east and west of the Niantic River and tidal studies indicated that winter flounder larvae from those sites entered Niantic Bay from Long Island Sound. In our study, mass-balance calculations were used to investigate whether the number of larvae entering Niantic Bay from the Niantic River could sustain the number of larvae observed in the bay during the period from 1984 through 2001. Further, we estimated the number of entrained winter flounder larvae originating from the Niantic River and compared these results with estimates derived from genetic stock analysis performed in 2001. The lowest percent entrainment attributed to the Niantic River winter flounder stock was observed in 1997, with 12.3% of 9.3 million larvae entrained originating from the Niantic River. The highest percent entrainment attributed to the Niantic River stock occurred in 1996, when 58.8% of the 30.4 million entrained larvae were determined to be of Niantic River origin. In 2001, mass-balance calculations and genetic stock analysis provided similar results, determining that 21.4% and 22%, respectfully, of the 80.7 million larvae entrained were of Niantic River origin. Both methods showed that peak fractional entrainment of Niantic River larvae occurred early in the spring. Together, these studies provide a validated characterization of the sources of winter flounder larvae entrained at MPS.

The Enigmatic Deep-water Witch Flounder of the Mid-Atlantic Bight

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The existence of a deep-water (greater than 366 meters) resource of witch flounder, *Glyptocephalus cynoglossus*, along the northeastern U.S. continental slope and adjacent abyssal plain is suggested by several lines of evidence including: 1) egg and larval distribution patterns; 2) by-catch rates in deep-water surveys for red crab (*Chaceon quinquedens*) and monkfish (*Lophius americanus*); and 3) various special deep-water studies conducted as far south as Virginia. Nothing is known regarding the abundance, biology, and production rates of these fish or their affiliation to witch flounder in shallower shelf waters. Recent opportunistic sampling at depths ranging from 367-914 meters has provided a limited number of samples for the preliminary estimation of growth and maturation rates. When compared to witch flounder of the shallower regions of the Gulf of Maine and Georges Bank, growth rates for deep-water fish were considerably lower and maturation occurs at a greater age. Differences in otolith morphology and length-weight relationships were also observed. Possible linkages of these deep-water witch flounder to other populations in the Northwest Atlantic are hypothesized.

Culture of Atlantic Halibut (*Hippoglossus hippoglossus*) in Offshore Net Pens

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The University of New Hampshire's Open Ocean Aquaculture Demonstration Project seeks to stimulate the development of commercial aquaculture in New England. Among the finfish species we have been working with, Atlantic halibut were selected because of their high market value and demand, tolerance to cold water, good growth rates and feed conversion ratios, disease resistance, and reduced availability from the wild fishery.

In May of 2001, 2000 juvenile halibut (30 g mean weight) were purchased from R&R Development Ltd. in Digby, Nova Scotia, and transferred to the UNH Coastal Marine Lab in New Castle, NH. Here, the fish were cultured to 100 g mean weight in a flow through seawater system. In October of 2001, the halibut were transferred to a 600 m^3 Sea Station cage located 14 km offshore in 52 m water depth. The cage was submerged 12 m below the ocean surface for grow-out. Fish have been fed (Shur Gain™ halibut diet) once per day at a rate of 3-4% body weight. Fish are being sampled once per month for survival, weight, and total length.

Survival has been excellent (>90%), and the current average weight of the fish is 456g. We expect to harvest them when they reach 1-2 kg, which should occur in the summer of 2003. The only difficulty we have encountered has been with fat cell necrosis syndrome ("sunburn") in some mal-pigmented fish maintained in a surface cage. Information gained from this initial growout experiment will hopefully further the development of halibut culture in New England.

Abstracts
Poster Presentations

Squamation, Pigmentation, and Asymmetry in Summer Flounder

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Flatfishes, such as the summer flounder (*Paralichthys dentatus*), provide unique opportunities to study the development of morphological asymmetry. We have examined left/right differences in squamation and pigmentation throughout early development in *P. dentatus*, documenting scale development and location in relation to age, size, and overall pigmentation pattern (normal, albino, or ambicolored). General patterns of scale development in *P. dentatus* resemble those in Japanese flounder (*P. olivaceus*; Seikai, 1980). In both species, scale development progresses from posterior to anterior, and from the center of each side out toward the dorsal and ventral margins, with squamation more advanced on the ocular side than on the blind. In malpigmented summer and Japanese flounder, pigmentation is associated with the degree of ctenoid scale development: dark pigmentation correlates with the presence of ctenii. Kikuchi and Makino (1990) noted that in ambicolored Japanese flounder (*i.e.*, those with a partially dark blind side), pigmentation and ctenoid scales appear to "wrap" around the dorsal and ventral margins from the ocular to the blind side. We see the same pattern in ambicolored summer flounder. The association of scale type with pigmentation – ctenoid scales in dark areas of skin, and cycloid scales in light areas – appears essentially the same in summer as in Japanese flounder. However, our observations of radii, in all summer flounder scales we have examined, suggest that tentatively identified "cycloid" scales may actually represent primary (incompletely developed) ctenoid scales. This raises the question of whether there are any true cycloid scales in juveniles of this species.

The California Halibut as an Aquaculture Candidate: A Comparison to Other Flatfish

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Research into the culture of the California halibut, *Paralichthys californicus,* is comparatively new but has the advantage of building on information gathered for other flatfish aquaculture candidates. This presentation compares what information is available for the California halibut to that for other flatfish species. As with other flatfish, a combination of rotifers followed by enriched brine shrimp nauplii and some formulated feed were initially used for rearing California halibut larvae. The employment of a static green-water type system in the second season of research dramatically increased the numbers of larvae brought through metamorphosis. Substantial improvements in weaning were also made during the second year of the project in which weaning was achieved as early as 42 days, compared to over 100 days in the first year.

The characteristic morphology and behavior of metamorphosed flatfish offer a number of advantages to culturists. All the flatfish being considered for culture are temperate species but increases in growth rate are noted with limited increases in temperature. A summary of growth rates (Specific growth rate; SGR, %/day) for a number of flatfish species with notes on culture conditions and feed is presented. Work to date with the various flatfish species suggests, as with other marine species, a high requirement for dietary protein. Although lipid requirements have yet to be defined it is thought they will most easily be satisfied through the use of marine lipids. Conversely, meeting these requirements for protein and marine lipids undoubtedly will be expensive. Fortunately, in that these fish spend much of their time lying motionless on the bottom, food conversion should be particularly attractive. In addition, provision of inputs such as oxygen and removal of wastes should be less of a challenge that for actively swimming species. It appears that culture density of flatfish, as defined by coverage of the tank bottom, can be surprisingly high. Some species have been grown at densities over 100%, which necessitates the fish stack on top of each other. One other particularly interesting component of flatfish culture is the potential for various flatfish to be cultured in an environment of reduced salinity. This could allow culturists to move away from expensive coastal sites. Information available with regard to growth and survival of various flatfish species under reduced salinity conditions is summarized. While we are still in the process of researching many of the above culture components, work to date suggests that the California halibut has the potential to become one of a number of flatfish species that will be commercially cultured in the future.

Initial Evidence of Vertical Migration of Winter Flounder (*Pseudopleuronectes americanus*) in a New Jersey USA Estuary

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Our prior research indicated that coves near Little Egg Inlet, New Jersey are settlement areas for winter flounder (*Pseudopleuronectes americanus*), and that estuarine circulation patterns in this flood-dominated system supported the advection of larvae into these coves. The purpose of the present study was to determine the vertical distribution of larvae. We performed both surface and bottom plankton tows (bongo nets) synchronously with repeated Acoustic Doppler Current Profiler (ADCP) transects over the study area. Despite tidal currents approaching 2 m/s, our observations indicate that the temporal variability of larval abundances cannot be explained solely on horizontal advection. On average, more larvae (42-489.8/1000m^3) were collected at the bottom than at the surface (0-233.0/1000m^3). We always collected larvae at the bottom, but collected no larvae at the surface 31% of the time. These results, in conjunction with results from simultaneous 1-m plankton tows, indicate the importance of behavior in the advection of these larvae into settlement coves. Larvae that migrate to the bottom of the channel during the ebb may avoid being swept out the inlet and instead may move to the surface during the subsequent flood to find suitable settlement habitat within the estuary.

The Contribution of Southern Flounder and Summer Flounder to the Recreational Fishery of the Southeastern USA

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The Marine Recreational Fisheries Statistics Survey (MRFSS) program of the National Marine Fisheries Service (NMFS) is a national program designed to estimate marine recreational finfish total catch, and angler effort and participation. It consists of a voluntary intercept survey of fishermen upon completion of their fishing trip and provides information on species catch and associated lengths and weights. The purpose of the present study is to demonstrate the value of the MRFSS program in providing important data to fisheries biologists despite the relatively small number of fishes collected. Between 1997 and 2001, the combined catch of the summer flounder, *Paralichthys dentatus*, and the southern flounder, *Paralichthys lethostigma,* contributed up to 8% of the recreational total catch in North Carolina, 8.1% in South Carolina, 2.9% in Georgia, and 4% in eastern Florida. However, North Carolina was the only state in which summer flounder dominated the flounder catch. The contribution of flounder ranged from a low of 1.3% (GA, 1997) to a high of 8.1% (SC, 2000). In 1998, a 12-inch size restriction was placed on flounder and we investigated the impact of this regulation. Using only data provided by DNR in Georgia for southern flounder, there was a significant difference in the fish sizes collected over the years. In 1997, 42% of the fish were under 12 inches, but only 10% were undersized in 2001. This encouraging news indicates that recreational fisherman seem to follow guidelines regarding size restrictions.

Mortality of YOY Winter Flounder Held on Newark Bay Sediment: Some Unplanned Observations

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Young of the year winter flounder (*Pseudopleuronectes americanus*) from Sandy Hook Bay suffered unexpectedly high mortality (45% in seven days) when held on sediments from a shallow area (0.5 m MLW) of Newark Bay off Bayonne NJ. Fish were randomly allocated to tanks containing either clean sand or Newark Bay sediment for the purpose of generating treatment and control animals to competitive predation experiments. Tanks were well flushed resulting in a maximum dissolved oxygen concentration gradient of less than 6 μM (0.2 mg/L) from inlet to outlet. Mortality was minimal among controls on clean sand, but in the tank containing Newark Bay sediment some fish were found dead on top of sediment and many more had died while buried, suggesting the latter had not made an effort to escape the lethal conditions. The shallow depth (3 cm) of sediment employed and the measures taken to preclude hypoxia in the water suggest that constituents of Newark Bay sediment other than biogeochemicals were responsible for the mortality.

Abundance, Distribution, and Condition of Hogchokers (*Trinectes maculatus*) in the Hudson River Estuary, April – September 2002

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Hogchokers (*Trinectes maculatus*) are an abundant component of estuarine systems in the mid-Atlantic Bight. Despite their abundance, little is known about hogchoker biology and their trophic role in general. This study was undertaken to provide new information on aspects of abundance, movement, growth, and reproduction of hogchokers in the Hudson River estuary. Hogchokers were collected during monthly bottom trawl surveys of the Lower Hudson River estuary during the spring through autumn of 2002. Fish were collected during 5-minute tows of two otter trawl sets at each of 10 stations spanning from lower Manhattan (Battery) to Newburgh, New York. Abundances and location of captured young-of-the-year, older juveniles and adults were recorded. These fish were processed at the laboratory for body length and weight, gender, and liver and gonad weights. The liver and gonadal weights were used to compute hepatic-somatic and gonadal-somatic indices, respectively, in order to identify spawning time and to define the role that the liver might play in fish condition and reproductive effort. Results of this study will be compared with earlier results from the Hudson River and Delaware Bay.

Temporal Changes in Behavior of the Blackcheek Tonguefish, *Symphurus plagiusa*

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Blackcheek tonguefish, *S. plagiusa*, are abundant in shallow coastal waters and have insignificant value as a sport fish or for the commercial fishing industry. In order to assess the behavioral patterns of one of the least understood flatfishes, observations were performed over a 48-h period in a controlled environment. Individuals were caught via trawl, measured, and randomly placed in separate experimental chambers (84 x 43 x 34 cm) within 36 h of capture. Chambers contained 1 cm of clean graded sand and 10 cm of water (30°C and 29 ppt at start of experiment). To determine if any diel or tidal rhythmicity existed, fish were observed in approximate 3-h intervals in correspondence with high, ebb, low, and flood tide. A light with a red filter was used at night to minimize disturbance. Diel activity was quantified by noting the percent of the tonguefish body covered by sediment. That, in conjunction with data regarding whether individuals changed orientation or changed location, was utilized to determine activity patterns. Preliminary results suggest that blackcheek tonguefish were more active at night as indicated by less sediment coverage and the fact that fish changed location frequently. Future studies will focus on whether interaction between tidal stage and light level affects tonguefish movement patterns.

Site Fidelity Patterns of Sub-legal Summer Flounder in Virginia Waters from Angler-assisted Tagging Program Data, 2000-2002

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Summer flounder, *Paralychthys dentatus*, ranks among the top finfish landed in both Virginia's commercial and recreational fisheries. Under the Summer Flounder Fishery Management Plan, incremental increases of recreational fishery minimum size limits in 2002 to 394 mm TL (VA's coastal fishery) and 444 mm TL (VA's Chesapeake Bay fishery), coupled with angler bag limits and 1-2 week mid-summer fishery closures, are contributing to rebuilding of the stock. Requiring anglers to release greater proportions of their catches also provided an opportunity to learn more about the distribution and habitat use patterns of 1-3 year old flounder through the Virginia Game Fish Tagging Program (VGFTP). Beginning in 2000 the VGFTP has tagged over 10,800 juvenile flounder (through July 2002; fish typically 279-381 mm TL). Approximately 900 recaptures have been reported (8 % return rate). Fish are tagged in the caudal peduncle musculature with a Hallprint T-bar tag (anchor-10-mm long; sheath 65-mm long), a tag having approximately100% retention and producing no significant tagging mortality (from 3-10 day cage trials with tagged fish). Most tagging, and subsequent recaptures, occurred at fishing piers, bridge-tunnel complexes, and jetties. At several locations in 2001, 7-15 multiple recaptures (2-5 recaptures of the same tagged fish) provided unique data showing that some fish remained in the vicinity of certain structure sites over significant periods of time, *i.e*., May through July/August and September-November. For example, a fish was recaptured on three separate occasions at the same pier between June and August (serial periods at large of 32/44/and 67 days); similarly, a fish recaptured five times at the same pier demonstrated site fidelity over serial periods of 12/13/50/58/and 74 days. At another pier a tagged fish was recaptured three times at the site during September-November (serial periods of 2/19/and 64 days). Recaptures of fish tagged during one year and recaptured the next year also demonstrate some occurrences of year-to-year site fidelity, both for sites in lower Chesapeake Bay as well as sites around ocean inlets. Short and long distance movements of flounder have also been demonstrated. Recaptures of flounder tagged at the Chesapeake Bay mouth have occurred from off Cape May (NJ), Long Island Sound (NY), North Carolina, and South Carolina beaches, a general pattern observed in earlier VIMS studies (1987-89 and 1995-96).

Embryonic and Larval Staging of Summer Flounder, *Paralichthys dentatus**

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Summer flounder, *Paralichthys dentatus*, is an increasingly important aquaculture species in the U.S. Like most other marine fishes, however, its early development is poorly known, mainly due to the historical difficulty of obtaining pelagic embryonic and larval stages. The cooperation of a commercial hatchery has enabled us to prepare a detailed staging table for embryonic and larval development using large numbers of embryos of known ages reared under controlled conditions. Our staging scheme is designed to facilitate rapid assessment of developmental stage based on readily visible landmark features, while providing more detailed descriptions of the morphological differentiation of the jaw apparatus and digestive system. We divide development into two main periods, pre-hatching and post-hatching, each of which is further subdivided into discrete morphological stages. Pre-hatching stages (fertilization to hatching) are based loosely on Shardo's (1995) staging table for *Alosa sapidissima*; post-hatching stages (hatching to metamorphosis) are aligned with the staging table for Japanese flounder (*Paralichthys olivaceus*; Minami, 1982; Fukuhara, 1986). The appearance and increasing complexity of discrete morphological features provides a more reliable and less variable measure of development than do simple scalar measures such as length, width or age. This staging scheme should therefore facilitate accurate assessments of developmental stage, which are important both for a wide range of applied studies (for example on larval nutrition), and for basic descriptive and experimental research in this species.

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Seasonal Changes in Blood Chemistry of the Yellowtail Flounder, *Limanda ferruginea*

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Seasonal changes in blood chemistry and hematology were observed in yellowtail flounder, *Limanda ferruginea*, collected from the Northwest Atlantic. Variations in blood parameters appear to be regulated by seasonally-induced physiological and/or environmental factors. Plama osmolalities during the winter, spring and summer seasons were significantly greater than in the fall. Sodium concentrations peaked during the winter and declined during spring and fall. Potassium was highest in the summer and lowest during the winter. Calcium was significantly higher in fall than spring. Hematocrit was highest in the spring and lowest during the fall. Hemoglobin values were elevated during the winter and spring months and declined during the fall. This baseline collection of blood data may be useful in monitoring the health and condition of yellowtail flounder in nature or under aquaculture conditions.

Winter Flounder Stock Enhancement

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The decline of traditional ground fisheries gives urgency to aiding the crisis by augmenting indigenous populations with hatchery-reared fish. Winter flounder (*Pleuronectes americanus*) may be an excellent candidate for stock enhancement. From 1997 to the present, Llennoco, Inc. has been successfully spawning adult winter flounder, growing out juveniles and tagging winter flounder for stock enhancement. From 2000 to the present, Marine Research, Inc. has successfully transported, released and recaptured those hatchery-reared fish. Issues have been addressed and obstacles overcome in the process, particularly in the areas of maintaining broodstock, life support systems, food supply and distribution, monitoring systems, transportation methods, tagging, release and recapture. The hatchery methods utilized by Llennoco, Inc. have proven that it is economically and biologically feasible to produce millions of juvenile winter flounder at a reasonable cost. The procedures developed by Marine Research, Inc. have shown that the hatchery-reared winter flounder do survive following release in Plymouth and Duxbury Harbors. Recaptured individuals obtained by beach seine as much as 95 days post-release showed good growth rates and cage studies suggested that survival rates may be equal between tagged hatchery-reared fish and naturally occurring fish. Stomach content analyses showed that the diets of released hatchery-reared fish and wild fish were identical. As a result of these efforts, it appears that winter flounder may be used for large-scale enhancement program.

Winter Flounder (*Pseudopleuronectes americanus*) Spawning Areas in New Haven Harbor

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As part of a previous study in 1994, 24 gravid female winter flounder were fitted with acoustic transmitters and released in New Haven Harbor, CT prior to the spawning season. The transmitters sent out a unique numerical signal so that individual fish could be identified. We visited the harbor weekly during spawning season and systematically searched the harbor for tagged fish using a directional hydrophone. Areas where fish were found were thought to be potential spawning areas. Fish were frequently located in Morris Cove on the eastern side of the harbor and around the east breakwater. Using a benthic sled, we then sampled these areas in an effort to collect fertilized winter flounder eggs and to confirm the presence of spawning activity at the site.

Since only a few winter flounder eggs were collected in 1994, the same six sampling sites were sampled more intensively in 1997. Triplicate tows were conducted at each site approximately biweekly from February to the beginning of April. In 1999, four sites in the shallower areas of Morris Cove were sampled using the same protocol as for the six sites sampled in 1997. In total, over 1400 eggs were collected, 115 of which were winter flounder eggs. The greatest number of winter flounder eggs was collected in mid-March in both years. The observed distribution of winter flounder eggs indicates that the northern end of Morris Cove and the area adjacent to it and east of the main channel are spawning areas for winter flounder.

The Feeding Behavior of Atlantic Halibut, *Hippoglossus hippoglossus*

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Atlantic halibut, *Hippoglossus hippoglossus*, is currently being explored as an aquaculture candidate in coastal waters of northern New England. To gain a better understanding of the feeding requirements of this species, the following experiments were conducted.

The first experiment examined the effect of feeding frequency on growth, feed consumption and feed conversion ratio (FCR). Juvenile fish (20g; 20/tank) were reared at 13ºC in 140 l tanks in a closed recirculating system (n=three replicates/treatment) over an 84-day period. The fish were fed one, three and fives times daily over a 12-hour period under constant lighting. A significant difference in growth was observed between fish fed one and five times daily ($p < 0.05$). Fish fed once daily consumed significantly less feed during this study (731.7g) than those fed three or five times daily (856.3g and 888.7g, respectively). There was no significant difference in feed conversion ratio (FCR) among treatment groups.

A second experiment was conducted to determine if Atlantic halibut exhibit an increase in swimming activity prior to a scheduled feeding (Feeding Anticipatory Activity; FAA). The activity of the fish (n=10/group), entrained to one feeding per day, was monitored by video for 72-h periods. Video recordings of fish movement were digitized and converted to histograms for statistical analysis. Preliminary analysis did not confirm the existence of FAA under these experimental conditions. Additional feeding experiments are currently in progress.

Histological and Biochemical Comparison of Skin Development in Normal and Malpigmented Summer Flounder*

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We have applied two complementary techniques to document skin and pigmentation development in summer flounder: paraffin histology, which shows both general skin structure and the specific location of melanophores, and a biochemical (DOPA) assay that reveals melanophores at early stages of their differentiation. In newly-hatched larvae (stage A; Martinez and Bolker, 2002), the skin is not yet divided into distinct dermal and epidermal layers; this morphological distinction begins to appear at stage C, and is well developed by the onset of metamorphosis (stage F). No early melanophores are detectable by the DOPA assay at stage A, but scattered cells on both ocular and blind sides show a positive DOPA reaction by stage E (late premetamorphosis); this pattern continues through stage H (late metamorphosis). No DOPA-reactive cells are detectable at later stages. In metamorphosed larvae 50 days after hatching, a layer of melanin is present between the dermal and epidermal layers of ocular-side skin, but absent on the blind side. Scales and scale pockets, which extend deeply into the dermis, begin to develop by 75 days after hatching; melanin is localized mainly at the base of the epidermis on the ocular side. In juveniles (>60 mm), dermal melanophores gradually replace epidermal melanin as the primary basis of dark pigmentation on the ocular side, though some extracellular melanin remains in the epidermis. Malpigmented juveniles display the same skin structure and melanin distribution as normal individuals at the histological level; however, albino fish show typical blind-side morphology in ocular-side skin, and ambicolored fish have ocular-type skin and pigmentation on both sides of the body.

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Skeletal Anomalies in Offshore Species of Flatfish, American Plaice: A Comparison with Winter Flounder

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American plaice are a good choice as a sentinel species for habitat monitoring in the Gulf of Maine and Massachusetts Bay. They are widely distributed in deep water, their population is relatively stable, and they are susceptible to axial skeletal deformities which are readily imaged through X-ray analysis. Since 1992, plaice have been regularly collected on NEFSC groundfish cruises and brought to the Milford Laboratory for X-ray analysis where nine types of deformities have been documented: fusion of individual centra, complexed- vertebrae which have incompletely separated during embryogenesis, accessory processes, deformed centra, reduced centra, spinal curvature, reduced processes, and hyper-ossification.

X-ray analysis of a sample of 66 plaice collected in Massachusetts Bay near the Boston Effluent Outfall in spring 2002 revealed that 11 of 66 fish (16.6%) had vertebral deformities; the most common were fusion and complexed-vertebrae, which affected 7 of 11 fish. Eight of the 11 deformed plaice had evidence of hyper-ossification. Prevalence of deformities in the 2002 sample was compared with a similar sample collected during the fall of 1994 from Massachusetts Bay. It was also compared with axial skeletal deformities found on winter flounder from Boston Harbor and Georges Bank, collected during the period 1989-1995.

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