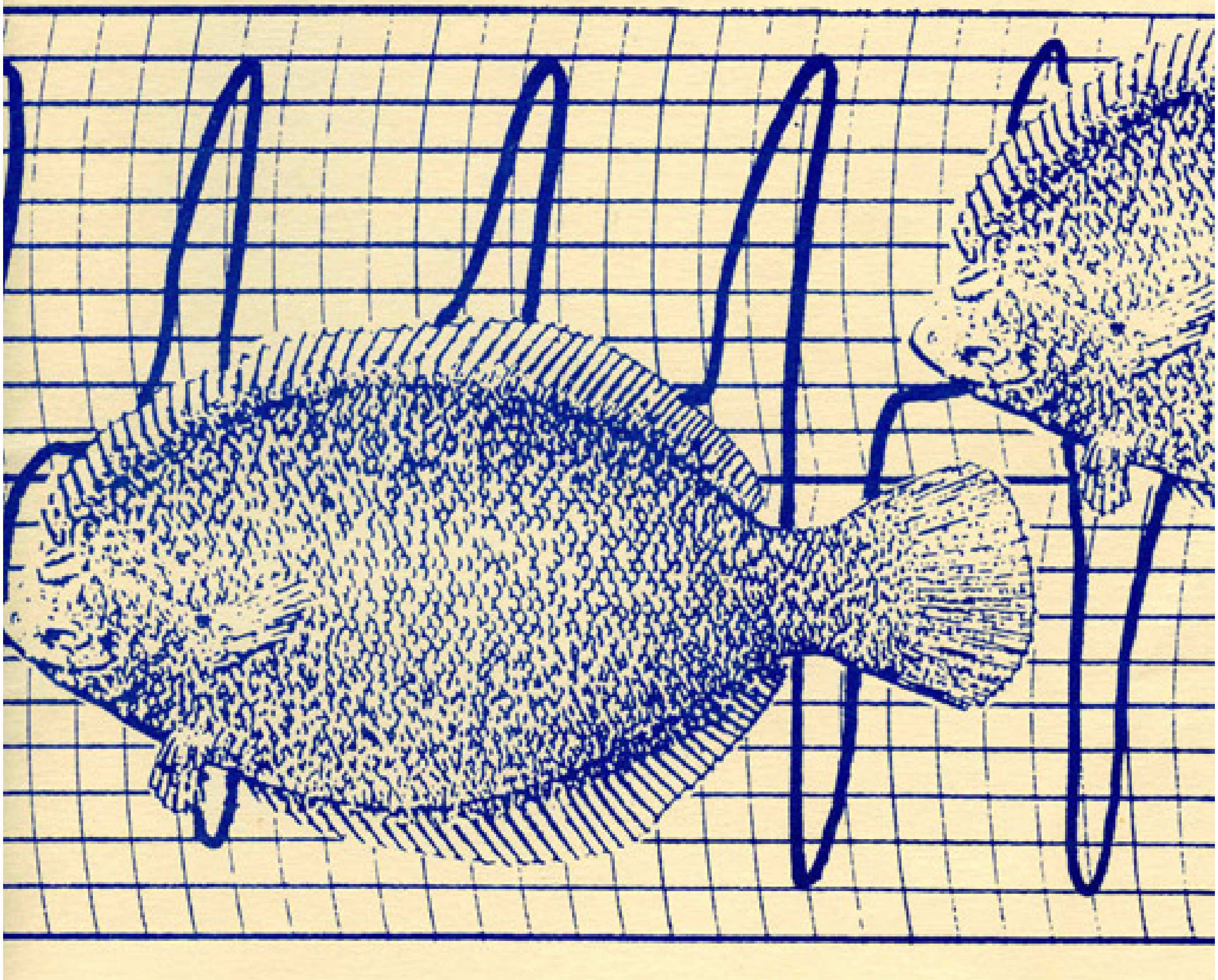
Workshop On

WINTER FLOUNDER BIOLOGY



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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 Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts

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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]

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Fort Valley, GA 31030

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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

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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.

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*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

R. E. Hillman, R. S. Carr, and J. M. Neff

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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

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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

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No Abstract