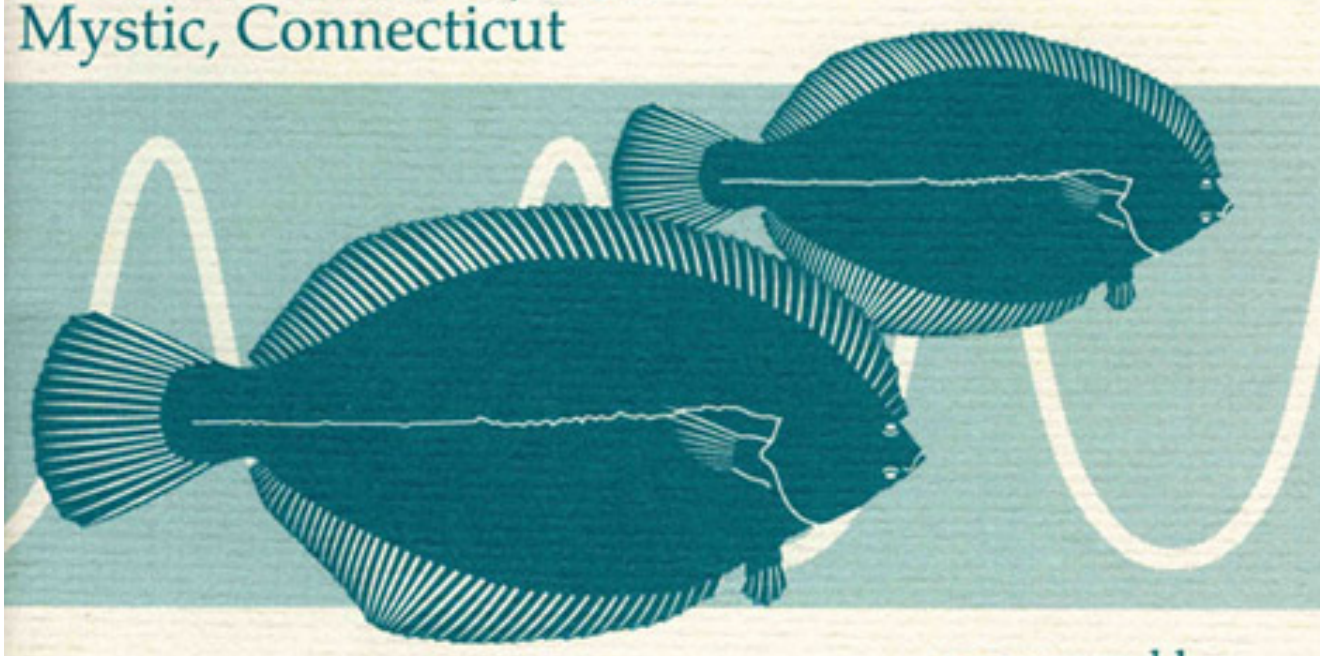
Workshop on Winter Flounder Biology

December 5 and 6, 1989
Mystic, Connecticut



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This document's publication history is as follows: manuscript submitted for review January 15, 2008; manuscript accepted through technical review February 6, 2008; manuscript accepted through policy review February 7, 2008; and final copy submitted for publication February 7, 2008. Pursuant to section 515 of Public Law 106-554 (the Information Quality Act), this information product has undergone a pre-dissemination review by the Northeast Fisheries Science Center, completed on February 6, 2008. The signed pre-dissemination review and documentation is on file at the NEFSC Editorial Office. This document may be cited as:

Calabrese A (chair), Beck A, Clark S, Danila D, Howe A, Howell P, Jearld A, Powell C, Studholme A. 2008. Workshop on Winter Flounder Biology, December 5-6, 1989, Mystic, Connecticut. US Dept Commer, Northeast Fish Sci Cent Ref Doc. 08-05b; 43 p. Available from: National Marine Fisheries Service, 166 Water Street, Woods Hole, MA 02543-1026.

Northeast Fisheries Science Center Reference Document 08-05b

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

February 2008

Acknowledgments

Prepared by
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831-6285
Operated by
Martin Marietta Energy Systems, Inc.
For the
U. S. Department of Energy
Under contract DE-AC05-84OR24100

Publication of the original booklet provided by the
Electric Power Research Institute
Under contract RP2932-2
with
Martin Marietta Energy Systems

Original Cover Design
Diane Rusanowsky
National Marine Fisheries Service, Milford, CT

Sponsored by

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

Northeast Utilities
and
Electric Power Research Institute

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
Storrs, CT 06268

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

R. E. Haas and C. W. Recksiek

University of Rhode Island
Department of Fisheries, Animal and Veterinary Science
Kingston, RI 02881

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*

A. Gupta and J. L. Renfro

University of Connecticut
Department of Marine Sciences
Storrs, CT 06269-3042

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.

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*Research supported by NSF.

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]

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Department of Chemistry and Chemical Engineering
Newark, NJ 07103

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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*Research sponsored by the Electric Power Research Institute under contract No. RP2932-2 (DOE No. ERD-87-672) with the U. S. Department of Energy, under contract No. DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

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.

*Research supported by CA44289 to A. E. McElroy and by a Lucille P. Markey Fellowship to A. E. McElroy and K. M. Kleinow.

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 x 10^{8} to 30 x 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)

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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)*

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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

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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

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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

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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.