·Dominion Nuclear Connecticut, Inc.
Millstone Power Station
Rope Ferry Road
Waterford, CT 06385
June 12, 2002

Dominion™

Info to ESSA on MBM

D17333

Mr. James Grier
Supervising Sanitary Engineer
Permitting, Enforcement & Remediation Division, Water Management Bureau
Department of Environmental Protection
79 Elm Street
Hartford, CT 06106-5127

References:
1. Email, J. Grier, Connecticut Department of Environmental Protection to P.M. Jacobson, Dominion Nuclear Connecticut, Inc., dated April 30, 2002 (10:07 AM).
2. Letter D04143, E.J. Mroczka to L. Carothers, dated October 30, 1990.
3. Letter D17249, W. Mathews to M.J. Harder, dated August 31, 2001.
4. Letter C09872, M.J. Harder to F.C. Rothen, dated February 16, 2000.
5. Letter D17306, G.W. Johnson to J.F. Grier, dated March 14, 2002.
6. Letter D17321, G.W. Johnson to A.J. Rocque, Jr., dated April 29, 2002.

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Millstone Power Station
Cooling-Water System Technology Study
Request for Information - Winter Flounder Mass-Balance Model

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Dear Mr. Grier:

By way of an email communication (Reference 1), the Connecticut Department of Environmental Protection (DEP) requested certain information from Dominion Nuclear Connecticut, Inc. (DNC) for use by its contractor, ESSA Technologies Ltd. (ESSA), for the purposed of performing a sensitivity analysis of the larval winter flounder mass-balance model. This model was developed at Millstone Power Station (MPS) and has been in use since 1990 (Reference 2). One of the most recent presentations of this model was in the final report on the use of once-through cooling water at Millstone Power Station (MPS), entitled "An Evaluation of Selected Cooling-Water System Alternatives for Millstone Power Station", which was submitted by DNC to DEP on August 31, 2001 (Reference 3). More specifically, the mass-balance model was described and a sensitivity analysis performed by DNC personnel in Appendix B to Chapter 3 of Part II of Reference 3. Also, please note that at the request of DEP (Reference 4) an independent review of this model was performed by Dr. Eric Adams of The Massachusetts Institute of Technology. This material was included as Appendix C to Chapter 3 of Part II of Reference 3.

Per your instructions, DNC has forwarded directly to ESSA the following materials:

1. SAS (Version 8.0 for Windows) disks to be used expressly for the purpose of performing a sensitivity analysis of the larval winter flounder mass-balance model. These disks should be returned to DNC as soon as possible.

2. The SAS program and two datasets needed to obtain results of the mass-balance model for the years 1984-99 (the years analyzed in Reference 3), copied onto a compact disc.

3. To aid in ESSA's review, the program (massbal2.sas) was annotated and a separate list of variables is included, detailing their function.

Also enclosed is the initial description of the mass-balance model as first submitted to DEP in October 1990 (Reference 2). In addition, portions of five Annual Reports (1983, 1984, 1985, 1988, and 1991) on the monitoring of the marine environment of Long Island Sound at MPS are included. To facilitate the review by ESSA, only relevant portions of the chapters on winter flounder studies describing special larval studies (e.g., import-export at Niantic River mouth) that provided information leading to the development of the mass-balance model were copied. Further included are copies of reports prepared by Drs. Joseph Crivello of the University of Connecticut and R. Bradley Moran of the University of Rhode Island on larval winter flounder stock identification studies by analysis of genetic and multi-elemental techniques, respectively, recently provided to DEP in Reference 5. These studies, which provide a direct measure of the proportion of the entrained larvae attributed to the Niantic River stock of winter flounder in 2001, were compared to findings of the mass-balance model in the latest annual report "Monitoring the Marine Environment of Long Island Sound at Millstone Power Station Waterford, Connecticut" (see pages 251-253), also recently submitted to DEP (Reference 6). A copy of relevant pages from this report is also included with this letter.

As there may be additional questions regarding these materials and to facilitate this review, DNC personnel are available to meet with representative of DEP and ESSA, if so desired. Please contact Mr. Paul Jacobson, Millstone Environmental Services at (860) 447-1791 ext. 2335 with any questions or to arrange such a meeting.

Very truly yours,

DOMINION NUCLEAR CONNECTICUT, INC.

Gerald D. Hicks
Director - Nuclear Safety and Licensing

Enclosures (reports cited above only)

cc (w/ all SAS materials and reports cited above):
Ian Parnell
ESSA Technologies Ltd.
#300, 1765 West 8th Avenue
Vancouver, BC, Canada V6J 5C6

cc (w/ reports cited above only):
Mr. Ernest Beckwith
Connecticut Department of Environmental Protection
Marine Fisheries Office
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The SAS program titled MASSBAL2.sas is a condensed and simplified version of the programs used by the Millstone Environmental Laboratory to estimate the proportion of entrained winter flounder larvae originating from the Niantic River. The simplified program combines all years (1984-1999) and provides output parameters necessary to evaluate the sensitivity of model input parameters. Model output will provide values comparable to previously reported mass-balance model outputs (e.g., Tables 38-40 of NUSCO (2000); however, actual values will not be identical due to: 1) the use of different time periods of larval occurrence between models (simplified model is constrained to discrete period for all years), 2) the assignment of the same stage-specific proportions to missing data for all years, and 3) the assignment of the same stage-specific daily mortality rates for all years (instead of using different values each year). Table 1 includes a list of variable names and definitions used in the SAS program.

Table 1

Input Variable From ESSA_1

sdate	sampling date
wdate	first day of sampling week
sta	sampling station, transformed to LOC (location)
rep	sample replicate (not needed)
stage	larval developmental stage (1-4)
den500	total WF larval density (no./500 m^3)
stden500	stage-specific density
mm1-mm12	stage specific densities for each 1-mm size class (not needed)

Working Variables

lden500	natural log-transformed total density (used to calculate weekly (wdate) means)
cumden	cumulative density, additive through season
days	counting variable, day of season

PROC NLIN Variables (Gompertz function) - also see Appendix B of Chapter 3 of Part II of DNC (2001)

A	alpha, asymptote estimate, index of cumulative density
T	time, in days, of inflexion point, peak larval abundance
K	shape parameter
pred	predicted value at any point during season
inflex	predicted value at time T (not really needed unless you want to plot Gompertz curves)

More Working Variables

group	first date of 5-day sampling period
mngroup	stage-specific mean for each GROUP (5-day period)
sumstage	total mean for each GROUP
stagepro	stage-specific proportion (fraction of larvae represented by each developmental stage)
prost1-prost4	STAGEPRO renamed for each stage, essentially turning 4 observations per GROUP (1 per stage) into 1 observation with 4 variables

Input Variables From ESSA_2

sdate	sampling date
year	4-bit year
tvol	total daily cooling water volume (x 10^6 m^3)

Additional Flow-related Working Variables

totflow	"expanded" TVOL, daily cooling water volume (m^3)
meanflow	mean daily volume by GROUP (5-day period)

Remaining variables are defined in SAS code or in winter flounder mass-balance Materials and Methods section of any recent report, or DNC (2001).

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Northeast Utilities Service Company
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survey. During tagging operations, winter flounder larger than 20 cm were sexed, scales removed for aging, and length recorded to the nearest mm. A white 1.3-cm diameter disc uniquely numbered and printed with information for its return was positioned on the nape of the right side of the fish and a red disc with additional information was used on the left side. A nickel pin was pushed through the musculature, cut to size, and its end was crimped over to connect the tags and hold them in place. Except for some specimens released specifically at the MNPS intakes, winter flounder were returned to the same location as their capture. Information requested at recapture included date, location, method of capture, length, sex, and additional scales. A reward was given to all persons returning a tag.

Early life history studies

Larval stage

Samples examined for winter flounder larvae were taken at the MNPS discharge (station EN, formerly designated as DIS; NUSCo 1983); at Station NB in mid-Niantic Bay (formerly NB 5); and at stations A (new in 1983), B (realignment of former station NR 1), and C (formerly NR 2) in the Niantic River (Fig. 2). Entrainment samples at EN were collected on
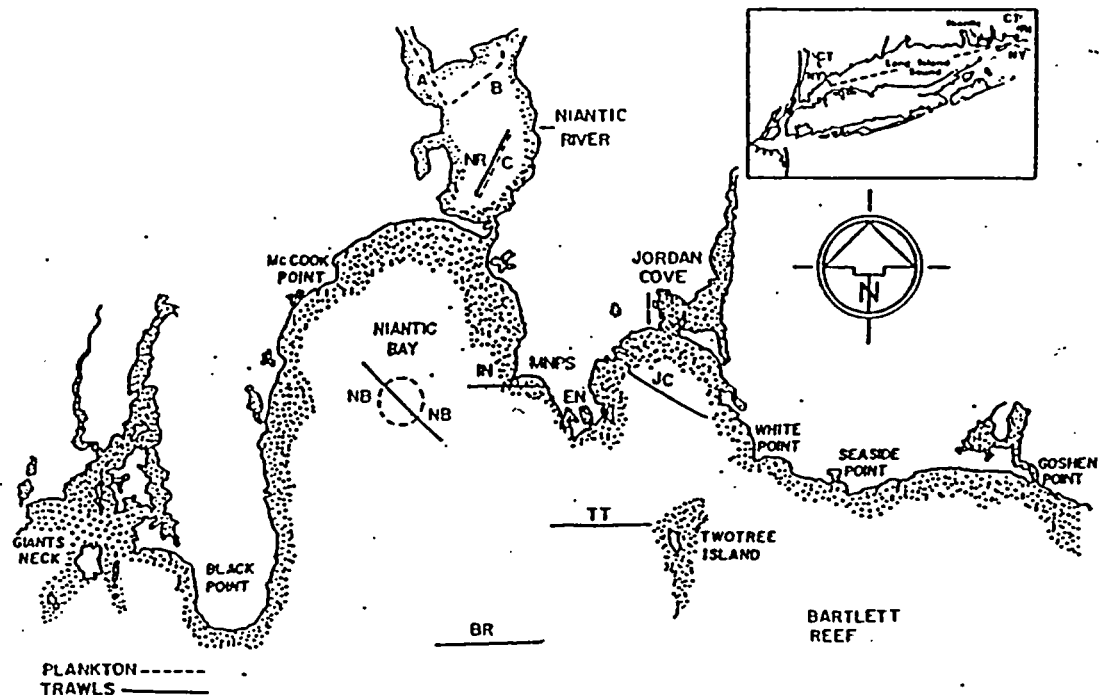

Figure 2. Location of stations sampled for winter flounder in the trawl and ichthyoplankton monitoring programs.

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4 days and 4 nights each week, alternating weekly at the discharge of Units 1 and 2. Approximately 400 m^3 of water were filtered through a 1.0-m diameter, 3.6-m long, 0.333-mm mesh conical plankton net. Additional details may be found in the Fish Ecology section.

Ichthyoplankton samples were taken in Niantic River and Bay with a 60-cm bongo sampler with 3.3-m long nets of 0.333-mm mesh towed at 2 knots and weighted with a 28.2-kg oceanographic depressor. Volume filtered was determined with General Oceanics flowmeters (Model 2030) and approximated 300 m^3 per sample. Single tows (one replicate processed) were taken both day and night using a stepwise oblique tow pattern with equal sampling duration of 5 min in surface, midwater, and bottom strata. The length of tow line necessary to sample the mid-water and bottom strata was based on water depth and the tow line angle as measured with an inclinometer and was determined by the following relationship:

tow line length = desired sampling depth/cosine of tow angle.

Tow duration was reduced to 2 min per strata at station A starting on May 28 due to net clogging by lion's mane jellyfish (Cyanea sp.). At NB, single bongo tows (both replicates processed) were made biweekly from January through March. From April through the end of the larval winter flounder season in mid-June, single bongo tows (one replicate processed) were taken twice weekly (Monday and Thursday or Tuesday and Friday) during day and night. In the Niantic River, preliminary tows were made during the day in February at stations A, B, and C at about weekly intervals to determine when larval winter flounder were present. From March through the disappearance of larvae at each station, single bongo tows (one replicate processed) were made twice weekly (Monday and Thursday or Tuesday and Friday) day and night.

Sampling time in the Niantic River during daylight and night was systematically varied between two daily periods (prior to 1200 and after 1200) and two nightly periods (prior to 2400 and after 2400) since the effect of time of day on collection densities was not known. No sampling was conducted 30 min before or after sunrise or sunset. The two daylight and two night sampling periods were alternated weekly.

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Station C was the only one in the Niantic River with strong tidal currents (Marshall 1960) and the effect of tide on collection densities was not known. Therefore, sampling at station C was systematically varied over four tidal stages (high, low, mid-ebb, and mid-flood). By collecting day and night samples approximately 6 h apart on one date, two opposing tidal stages were collected (e.g., high and low). The second weekly collection trip was 3 days later at approximately the same time and collections were taken during the other two tidal stages (e.g., mid-flood and mid-ebb). All 16 combinations of sampling periods and tidal stages were collected during every 4-wk period at station C. Two 24-h tidal studies were conducted at station C on April 28-29 and May 8-9. Samples were collected at 2-h intervals during a 24-h period.

Tidal export and import of larvae was examined at the mouth of the Niantic River during maximum ebb and flood currents. Two ebb and flood tides were sampled on May 9 and one ebb and flood tide was sampled on May 16. Stationary tows were taken in the middle of the channel adjacent to the Niantic River Highway Bridge. The bongo samplers described previously were used except an additional 40 kg of weight was added as ballast to increase the vertical tow line angle. Two bongo samplers were deployed for 15 min off each side of the boat with one at mid-water and the other near bottom. Two tows were made at each depth for a total of four replicates at each depth per tidal stage.

All ichthyoplankton samples were preserved with 10% formalin and processed in the laboratory. Samples were split to at least one-half volume and larvae identified and counted using a dissecting microscope. Up to 50 winter flounder larvae were measured to 0.1-mm in standard length (snout tip to notochord tip). The developmental stage of each larvae measured was recorded. The five possible stages were defined as:

Stage 1.   The yolk sac was present or the eyes were not pigmented (yolk-sac larvae)

Stage 2.   The eyes were pigmented, no yolk sac was present, and no fin ray development

Stage 3.   Fin rays were present but the left eye had not migrated to the mid-line

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Stage 4. The left eye had reached the mid-line but juvenile
characteristics were not present

Stage 5. Transformation to juvenile was complete and intense
pigmentation was present near the caudal fin base

Larval collection frequency and density ($n/500 \text{ m}^3$) were used for
data analyses. Collection frequency was adjusted for the number of
samples at each station and sample volume. Density distribution plots
were smoothed using the spline function (SAS Institute Inc. 1981).

Post-larval stage

During 1983, information was gathered on post-larval juvenile
winter flounder in the Niantic River. Four stations were sampled
including Sandy Point (SP), Lower River (LR), Camp O'Neill (CO), and
Channel (CH) (Fig. 1). SP, CO, and LR were selected because they had
good juvenile winter flounder habitat, with sandy to muddy bottoms in
shallow water adjacent to eelgrass beds (Bigelow and Schroeder 1953).
Station CH was in a slightly deeper area between stands of eelgrass and
the navigation channel. Depths sampled at all stations ranged from
about 1 to 3 m. The stations were sampled once each week from May 18
through October 12 during daylight from 2 h before to 1 h after high
tide. A 1-m beam trawl which had interchangable nets of 0.8-, 1.6-,
3.2-, and 6.4-mm bar mesh was used; the nets were changed as fish grew
and became available to the next largest size. A tickler chain was
added to the net for use with the three largest meshes. Three
replicates were made at each station and distance of each tow was
estimated by letting out a measured line attached to a lead weight.
Tows of 40 and 50 m made initially were increased to 75 and 100 m as the
number of fish decreased throughout the summer and early fall. For data
analysis and calculation of CPUE, the catch at each station was adjusted
to 100 m^2 of bottom covered by the beam trawl.

Juveniles were measured to the nearest 0.5 mm in total length.
During the first 5 weeks of the study, standard length was also measured
as many of specimens had damaged caudal fin rays and total length could
not be taken. The relationship between the two was determined by a

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this size winter flounder change from a pelagic to a benthic habitat and thus were not susceptible to the plankton sampling gear.

The mean length at each station for different developmental stages provided additional insight into larval dispersion (Table 14). Although

Table 14. Mean length by stage of all measured larval winter flounder taken at stations in the Niantic River and Bay and at MNPS.

Developmental stage	Station	Number measured	Mean length (mm)	Standard error
1	A	239	2.7	0.02
	B	217	2.8	0.02
	C	171	2.7	0.02
	EN	29	2.9	0.10
	NB	11	3.1	0.10
2	A	480	3.4	0.03
	B	658	3.7	0.03
	C	655	3.8	0.03
	EN	974	4.3	0.03
	NB	780	4.3	0.03
3	A	64	6.5	0.11
	B	342	6.4	0.05
	C	646	6.4	0.04
	EN	1,333	6.1	0.02
	NB	605	6.4	0.03
4	A	14	6.6	0.20
	B	137	7.2	0.05
	C	255	7.4	0.03
	EN	599	7.3	0.03
	NB	210	7.6	0.05
5	A	0	-	-
	B	27	7.7	0.13
	C	67	7.8	0.10
	EN	149	7.8	0.07
	NB	23	8.7	0.57

Stage 2 larvae were fairly evenly distributed in Niantic River and Bay (Fig. 10), the lag in temporal occurrence in Niantic Bay (Fig. 11) was reflected in the larger mean lengths at station NB and EN. For Stages 3 to 5, the mean length was similar at all stations except NB, which had a larger mean length for Stages 4 and 5. Based on these data, it appeared that most of the dispersion from the Niantic River to Niantic Bay occurred during the Stage 2 developmental period.

Special studies

Primarily Stage 3 (62% of total) and 4 (30%) winter flounder larvae were collected during the two 24-h studies at station C. No apparent day-night relationship was found (Fig. 13). However, the bimodal cycle

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Results

over the 24-h period suggested a tidal influence. The tidal period observed during both studies was 12 h. A harmonic regression as described by Lorda (1983) using terms of sin(hours) and cos(hours) over a 12-h period with slack ebb occurring at hours 0 and 12 and slack high at hour 6 was fit to log-transformed $(n/500 \text{ m}^2 + 1)$ data (Fig. 14). The harmonic regression accounted for about 45% of the total corrected sums of squares (TCSS) with the two sampling dates combined.. Based on this model, collection densities increased on a flood tide with a peak prior to slack high and then declined during ebb tide. Analyses of covariance, with tidal effect as described by the sine-cosine function as the covariate, was used to examine sampling data and day-night effects (Table 15). An interaction term for sampling date by day-night effect accounted for less than 1% of the TCSS and was pooled with the error. The two sampling dates were significantly different and accounted for an additional 19% of the TCSS. In agreement with the 24-h plot (Fig. 13), the day-night effect was not significant. Weinstein et al. (1980) reported that three post-larval fish taxa (spot, Atlantic croaker, and Paralichtys spp. flounders) used vertical migration in response to tides as a retention mechanism in the Cape Fear River estuary. The day and night differences in frequency of Stages 3 and 4 larvae at stations B, NB, and EN (Fig. 10) showed that winter flounder larvae of these developmental stages were capable of vertical movements. At station C the lack of diel differences for Stages 3 and 4 larvae suggested a modification of behavior in response to tidal currents. The vertical movement from the bottom during during a flood tide would act as a retention mechanism in the Niantic River.

Table 15. Summary of analysis of covariance for 24-h diel study with harmonic components of the tidal effect used as covariates.

Sources	Sum of squares	% of total	
Tidal[a]	25.003	44.6	*[b]
Sampling date	10.882	19.4	*
Diel	1.986	3.5	ns
Model	37.871	65.5	
Corrected Total	56.087	—	

[a] - Includes both sine and cosine components

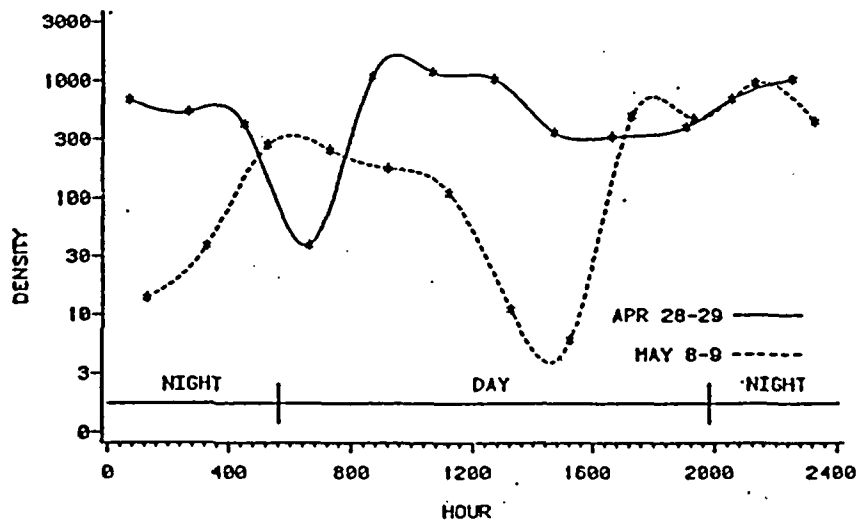[b] * - significant at $p \leq 0.05$
   ns - not significant

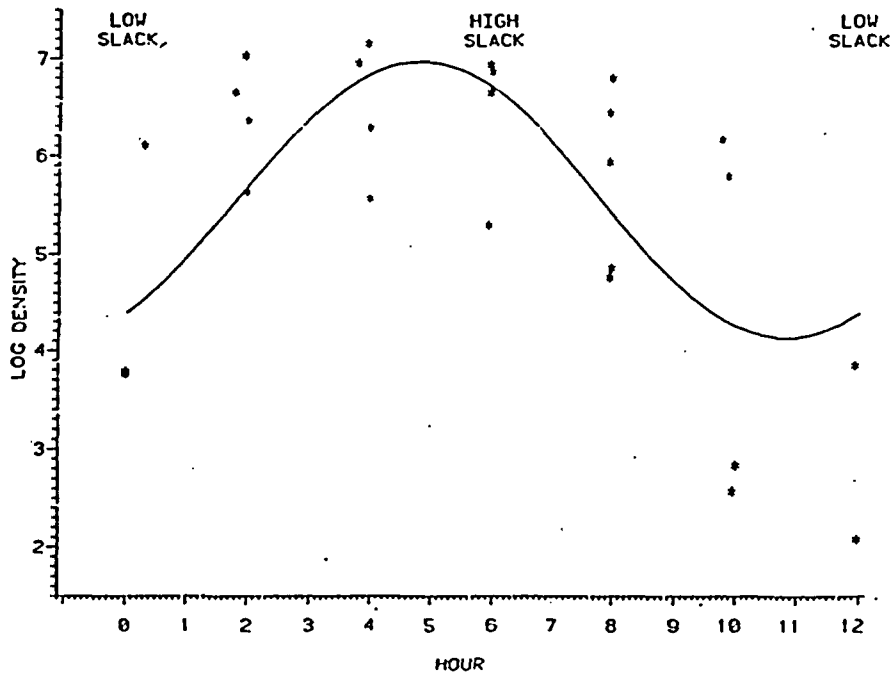Figure 13. Larval winter flounder density per 500 m^3 during the two 24-hr tidal studies at station C.

Figure 14. Larval winter flounder abundance (log density per 500 m^3) for the two tidal studies conducted at station C with the line fitted from the haromic regression (log density = 5.555 − 1.158 cos(hr) + 0.832 sin (hr)).

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The potential export or import of winter flounder larvae from the
Niantic River was investigated by sampling three ebb and three flood
tides at the river mouth. Most of the larvae collected during this
study were Stages 3 (45%) and 4 (48%). Many more larvae were collected
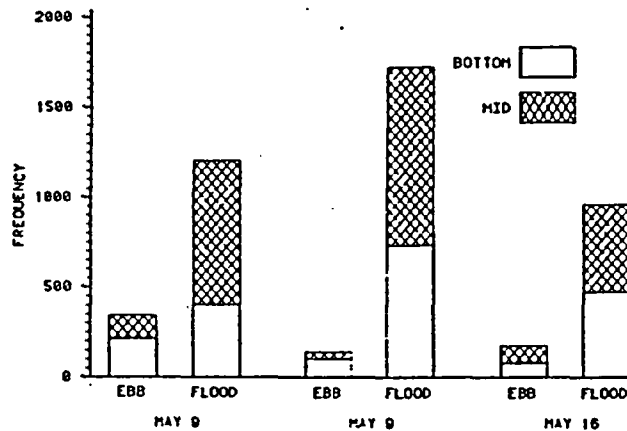during flood tide (Fig. 15). No consistent difference in collection

Figure 15. Frequency and collection times
of larval winter flounder taken
at the mouth of the Niantic River
during maximum ebb and flood tidal
currents.

frequency was found between mid and bottom depths. During the period of
sampling, there was a net increase in the number of winter flounder
larvae entering the Niantic River. The larval dispersion model for the
Niantic River (Saila 1976), which assumed larvae behaved as passive
particles, simulated an approximate 4% loss from the river per tidal
cycle. Stage 3 and 4 larvae, which have developing or developed fins,
may have used vertical movements in response to tidal currents for
transport into the Niantic River from the bay.

The abundance of lion's mane jellyfish (Cyanea sp.) medusae in the
Niantic River samples was measured volumetrically (Fig. 16). Volumes of
medusae increased at station A during late March and at station B during
early May. Marshall and Hicks (1962) also found that medusae were more
abundant in the upper river compared to the lower portion during May and
June. A peak occurred at stations A and B during mid-May. At station
C, jellyfish were most abundant in the last week of May. Although no

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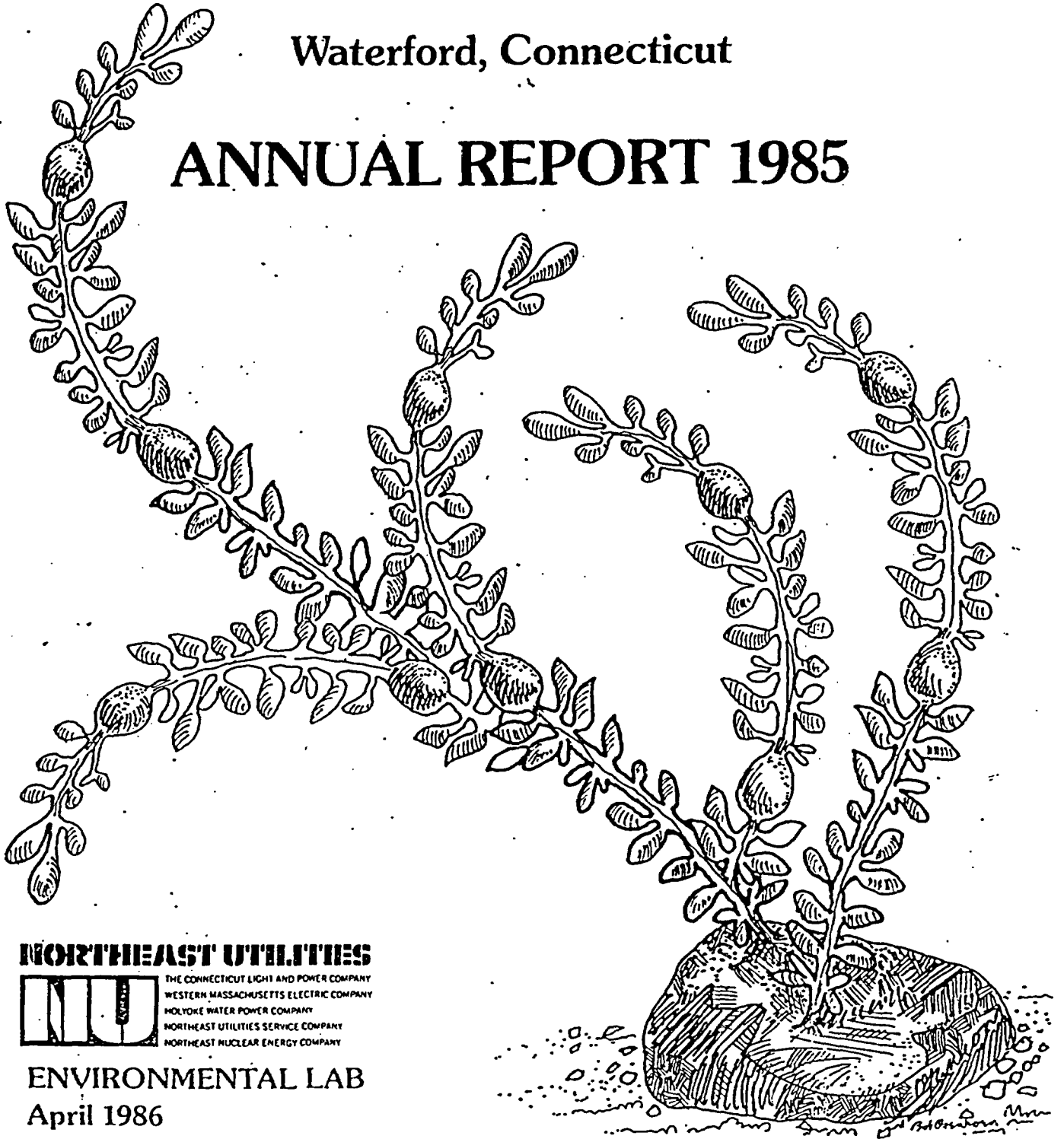
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ENVIRONMENTAL LAB
April 1986

corresponding to the inflection point of the cumulative function defined by its parameters (β) and (k) as:

$$t(i) = (\ln \beta)/k \qquad \qquad (6)$$

Least-squares estimates of these parameters were obtained by fitting Equation 4 to the cumulative abundance data using nonlinear regression methods (SAS Institute 1982).

Winter flounder larvae were reared in the laboratory during 1985 to determine developmental time and growth rate. Eggs were stripped from a female and fertilized with milt from two males. Larvae that hatched within 24 h of each other were placed in 39-1 aquaria held in a water bath. The water temperatures ranged from 4.3 to 9.1 C with a gradual increase during the holding period. Photoperiod was similar to natural conditions. Larvae were fed ad libitum rotifers (Brachionus plicatilus) and brine shrimp nauplii (Artemia salina). To examine the effect of starvation on growth, larvae in one aquarium were not fed. Known-age larvae were routinely sacrificed to obtain otoliths for aging verification and information on growth rate. Sampling frequency varied, with almost daily collections during early development to approximately biweekly during later development when the number of larvae remaining was low. Otoliths were prepared and examined in a similar manner as those collected in the field during 1984 (NUSCo 1985).

Larval import and export studies were conducted throughout complete tidal cycles on March 28, April 29, and May 28. Samples were taken hourly except between 1 h before and after slack tidal currents. Stationary tows were taken by mooring the boat to the Niantic River Highway Bridge in the middle of the channel. Bongo samplers with 0.202-mm mesh nets were used on March 28 and with 0.333-mm mesh nets on the other two dates. Bongo samplers were deployed off each side of the boat with one at mid-water and the other near bottom. Sampling duration varied from 6 to 15 min (depending on the current velocity) to sample approximately 100 m³ of water. Current velocity at the time of sampling was measured with a flowmeter mounted outside of the bongo opening so that back-pressure due to net clogging would not affect the measurement. These current velocities were used to calculate the net exchange of larvae leaving and entering the river.

Materials & Methods

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Ebb and flood tide velocity measurements used in estimating net larval exchange may not have been comparable due to the different widths of the channel at the point of sampling. Due to the length of the mooring line tied to the bridge, the actual sampling location was approximately 10 m north of the bridge during a flood tide and approximately 10 m south of the bridge during an ebb tide. The comparability of velocities was investigated by fitting a second order polynomial equation to the water velocity measurements over time during the three flood and ebb tidal phases sampled. The form of the equation was:

$$\text{velocity (cm/sec)} = at + bt^2 \qquad (7)$$

where t was time in h from high slack current for an ebb tide and from low slack current for a flood tide. No intercept was used because water velocity was zero at slack currents. For both tidal phases, a dome-shaped curve was formed that started and ended at zero velocity. The average duration in h of an ebb and flood tide was estimated by the root of the above equation (i.e., h after slack for the velocity to be zero again):

$$h = -2a/2b \qquad (8)$$

where h was the duration in hours and the parameters a and b were estimated separately for ebb and flood tides with Equation 7. Also, the area under the curves (i.e., an index of the total water volume during the sampling period) was determined by integration of Equation 7, which resulted in:

$$\text{area} = (a/2)(t^2) + (b/3)(t^3) \qquad (9)$$

Because fresh water input into the Niantic River is small, the volume of water leaving the river on an ebb tide should be similar to the volume that enters during a flood tide. Therefore, the areas under the curves should be similar. However, the area for the flood tide was less than the ebb. The flood tide velocities were recomputed by re-estimating the parameters (a) and (b) in Equation 7 under the constraint that the area for the flood tide be the same as the area for the ebb tide and using the known duration of the flood tide. This was done by solving for a in Equation 8 for the flood tidal phase (a = -hb) and substituting it into the area equation (9) of the ebb tidal phase where the t's were equated to the flood duration (h). This substitution caused Equation 9 to

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become a function of only the flood phase duration (h) and the parameter (b), so that:

$$\text{area} = -(1/6)bh^3 \qquad (10)$$

Since the area was also known, solving for (b) in the above gave a new estimate of (b); replacing this estimate in Equation 8 resulted in a new estimate of (a). The final step was the computation of the flow according to Equation 7, but using the new estimates of (a) and (b).

Post-larval Stage

Information on post-larval young-of-the-year winter flounder in the Niantic River was first gathered during 1983 (NUSCo 1984). One of the four stations established then, Lower River (LR), has been sampled through 1985 (Fig. 1). The other station sampled in 1985 was located near the Waterford shoreline (station WA) and was also sampled during August and September of 1984 (NUSCo 1985). Both stations contained habitat preferred by juvenile winter flounder, with sandy to muddy bottoms in shallow water adjacent to eelgrass beds (Bigelow and Schroeder 1953). The stations were sampled once each week from May 23 through September 19 during daylight within about 2 h before to 1 h after high tide. Depths sampled ranged from 1 to 2 m.

A 1-m beam trawl was used with interchangeable nets of 0.8-, 1.6-, 3.2-, and 6.4-mm bar mesh; a tickler chain was added to increase catch efficiency. Two nets of successively larger mesh were used during each sampling trip to collect the entire available size range of young. This helped to eliminate bias in the catch as was found in 1983, when some of the older and larger specimens apparently avoided the fine-mesh net needed to capture the smallest fish (NUSCo 1984). A change to the next larger mesh in the four net sequence was made when young had grown enough to become susceptible to it. The larger meshes also reduced the amount of detritus and algae retained. Two replicates with each of the two nets were made at both stations; the order in which the nets were deployed was chosen randomly. Distance of each tow was estimated by letting out a measured line attached to a lead weight as the net was towed. Tow length increased from 50 to 75 to 100 m as the number of fish decreased throughout the summer. For data analysis and calculation

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Tidal Import-Export Studies

Tidal import-export sampling at the mouth of the Niantic River was conducted on three dates in 1985. All winter flounder larvae collected on March 28 were Stage 1 and 2, on April 29 most (99%) were Stage 2 and 3, and on May 28 Stage 3 was dominant (89%). Examination of the combined data from all dates by percent occurrence of each developmental stage showed that Stage 1 and 2 larvae were more abundant during ebb tides and Stage 3 and 4 during flood tides (Fig. 13). Similarly, examination by
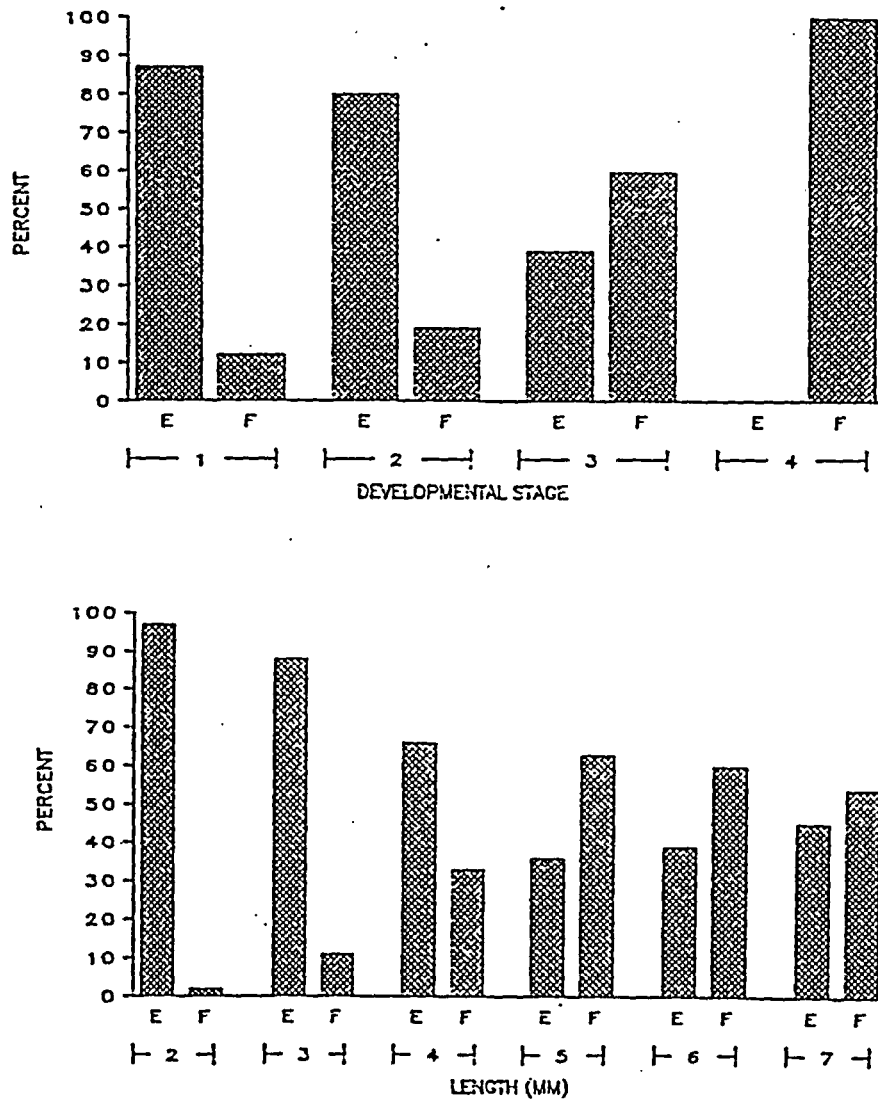

Figure 13. Percent occurrence of each developmental stage and 1-mm size class collected at the mouth of the Niantic River during ebb (E) and flood (F) tidal stages in 1985.

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1984

Northeast Utilities Service Company
April 1985

Sampling time and frequency varied with station and season and were partly based on information from the 1983 studies described in NUSCo (1984). At NB, single-bongo tows were made day and night biweekly from January through March. From April through the end of the larval winter flounder season in mid-June, single-bongo tows were taken twice weekly during day and night. In the Niantic River, preliminary tows were made during the day in February at stations A, B, and C at weekly intervals to determine when larval winter flounder were present. From March through the first week in April, single tows (not including additional tows to examine net extrusion) were made during the day twice weekly within 1 h of low slack tide. During the second and third weeks of April, single-bongo tows were made twice weekly day and night. The day samples were collected within 1 h of low slack tide and the night samples during the second half of a flood tide. During the remainder of the season until the disappearance of larvae at each station (last sample taken on June 14), tows were made twice a week only at night during the second half of a flood tide. Only one collection trip was made during the weeks of March 4 and May 27 because of adverse weather conditions.

The effect of tidal current on the collection of Niantic River winter flounder larvae and on their import and export were examined. Two 24-h tidal studies were conducted at station C on March 9-10 and March 18-19. Samples were collected at 2-h intervals during a 24-h period. Tow durations were 6 min and paired 0.202-mm and 0.333-mm mesh nets were used. Tidal import and export studies were conducted during two tidal cycles on April 4 and May 8. Samples were taken hourly except for 1 h before and after slack tidal currents. Stationary tows were taken in the middle of the channel adjacent to the Niantic River Highway Bridge. Bongo samplers with 0.333-mm mesh nets were used with an additional 40 kg of weight added as ballast to increase the vertical tow line angle. Bongo samplers were deployed off each side of the boat with one at mid-water and the other near bottom. Sampling duration varied from 6 to 15 min depending on the current velocity and approximately 100 m^3 of water was sampled. Current velocity at the time of sampling was measured with a flowmeter mounted outside of the bongo opening so that back-pressure due to net clogging would not effect the measurement.

see Fig 2 - follows

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Materials + Methods

These current velocities were used to calculate the net exchange of larvae leaving and entering the river.

Larval data analyses were based on density per 500 m³ and due to varying sampling frequencies data were reduced to weekly mean density. Data from all mesh sizes and tow durations were used in calculating the weekly mean densities. For comparisons, daylight samples in 1983 from the last week of April through the end of the season were excluded. These samples underestimated abundance because of diel behavior of the older larvae (NUSCo 1984). Daylight samples were not collected in 1984 during these weeks.

Most ichthyoplankton samples were preserved with 10% formalin. Except for tows made to compare net extrusion between 0.202-mm and 0.333-mm mesh nets, only one of the two bongo sampler replicates was processed for Niantic River and Bay samples. Samples were split to at least one-half volume and larvae were identified and counted using a dissecting microscope. Up to 50 winter flounder larvae were measured to 0.1-mm in standard length (snout tip to notochord tip). The developmental stage of each larva measured was recorded and the five stages were defined as:

Stage 1. The yolk sac was present or the eyes were not pigmented (yolk-sac larvae)

Stage 2. The eyes were pigmented, no yolk sac was present, and no fin ray development

Stage 3. Fin rays were present, but the left eye had not migrated to the mid-line

Stage 4. The left eye had reached the mid-line, but juvenile characteristics were not present

Stage 5. Transformation to juvenile was complete and intense pigmentation was present near the caudal fin base
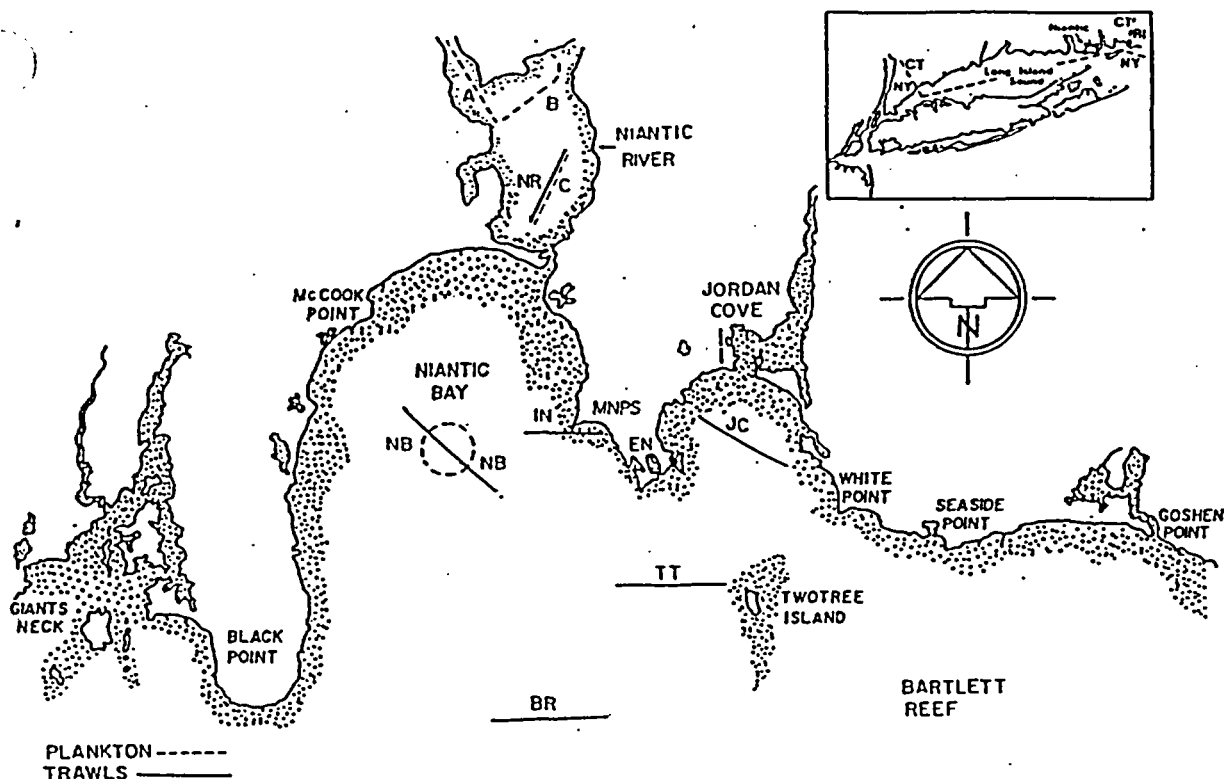
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Figure 2.   Location of stations sampled for winter flounder in the trawl
            and ichthyoplankton monitoring programs.

was based on water depth and the tow line angle as measured with an
inclinometer and was determined by the following relationship:

tow line length = desired sampling depth/cosine of tow angle.

From February 6 through March 19, 0.202-mm and 0.333-mm mesh nets
were paired on the bongo sampler and 28 samples were taken to compare
net extrusion between the two meshes at the Niantic River stations.   Nets
were towed for 6 min because the 0.202-mm mesh net clogged when duration
was greater.   After March 19, all collections were made with 0.333-mm
mesh.   When time permitted, consecutive 6- and 15-min tows were made at
the Niantic River stations with 0.333-mm mesh nets to compare net
extrusion for the two durations (16 paired comparisons).   A Wilcoxon
signed-ranks test was used to compare the paired samples and test for
significant differences due to mesh and tow duration.   Beginning in
April at stations A and B, all tows were 6 min due to clogging by Cyanea
spp. hydromedusae.   At station NB, 0.333-mm mesh nets and 15-min tows
were used throughout the season.

size-classes showed that larvae 4 mm and smaller were more abundant during an ebb tide and larvae 5 mm and larger were more abundant during a flood tide.

In order to determine if velocity measurements were comparable between ebb and flood tides, separate quadratic polynomial equations were fitted to hourly velocity measurements combined from each of the three ebb and flood tides sampled. Good fits were obtained for both ebb ($R^2=0.98$) and flood ($R^2=0.97$) tide equations (Table 15). The mean

Table 15. Quadratic equations used to describe ebb and flood tide velocities (cm/sec), with estimates of tidal stage durations, integrated areas under the velocity equation curves, and final equation to adjust flood-tide velocities (see text).

	Ebb	Flood
Actual (Eq. 7)[a]	Vel = $52.6(t) - 7.4(t^2)$	Vel = $5.17(t) - 8.8(t^2)$
Duration (Eq. 8)	6.9 h	5.8 h
Area (Eq. 9)	417.3	294.1
Adjusted velocity[b]	–	Vel = $73.3(t) - 12.6(t^2)$

[a] Equations found on page 11.

[b] Based on re-computed parameter estimates of (a) and (b) from Equations 8 and 10 (pages 11 and 12).

duration of each ebb tide was about 1 h longer than the flood tide and the area under the curve for the latter was smaller than the former. This difference in area indicated that if the flood sampling had been conducted at the same location as ebb sampling, the flood velocities would have been higher. Therefore, the flood velocities were estimated by re-computing the parameters (a) and (b) with Equations 8 and 10 and then substituting them into Equation 7. The calculations of net exchange of larvae which follow were based on actual ebb current velocities and the adjusted flood current velocities.

Using data combined from the three sampling dates, net tidal exchange was estimated for each 1-mm size-class; the 2- and 3-mm size-classes were combined because of the low collection densities of the former. The estimates were obtained by summing the number ($n/500$ m^3) of larvae of each size-class in each hourly sample for the three sampling dates. The sum was multiplied by the estimated water velocity at the

time of the hourly collection. This density-velocity adjustment accounted for changes in discharge volume during the tidal cycle. Since larvae collected during an ebb tide represented a loss from the river, the density-velocity value was made negative. A harmonic regression equation using a 12.7-h tidal cycle (the average duration of the three tides sampled) was fitted to density-velocity values. The area under the curve for each tidal stage was estimated by numerical integration of the harmonic regression equation using 5-min increments. Net tidal exchange was expressed as the percent return of a size-class on a flood tide compared to the loss on a ebb tide (Table 16). There was a net export of 4 mm and smaller size-classes and a net import of 5 mm and larger size-classes.

Table 16. Percent return of larval winter flounder on a flood tide that were flushed from the river on an ebb tide by size class and R^2 values of the harmonic regression models.

Size class (mm)	Percent return	R^2 of model
3	19.8	0.81
4	48.8	0.52
5	174.9	0.91
6	136.8	0.82
7	103.8	0.66

The 1985 import-export data were consistent with previous findings (NUSCo 1984, 1985). Since 1983, eight tidal cycles were sampled and these data clearly indicated a net loss of smaller larvae that lack fin ray development and have little or no locomotion. However, larger larvae with developed fin rays apparently utilized vertical migration in relation to tidal currents for passive migration back into the Niantic River. This vertical migration of larvae after fin ray development was also apparent during 24-h studies conducted in the river in 1983 (NUSCo 1984), where densities of larger larvae increased during a flood tide and decreased during an ebb tide. Vertical migration was not apparent during 24-h studies in 1984 (NUSCo 1985) since all sampling was conducted prior to fin ray development. Other researchers have also

reported vertical migration in early life history stages of fish. Diel movement of larval yellowtail flounder was found to increase with the size of the larvae (Smith et al. 1978). Atlantic herring larvae synchronized vertical migration with flood tides to minimize seaward transport (Fortier and Leggett 1983). Post-larval spot, Atlantic croaker, and Paralichthys spp. flounders used verical migration in response to tides as a retention mechanism (Weinstein et al. 1980). Larval North Sea plaice demonstrated selective horizontal transport by swimming up from the bottom during flood tides and remaining near the bottom during ebb tides (Rijnsdorp et al. 1985). Most winter flounder larvae found in Niantic Bay probably were tidally flushed from the Niantic River during early developmental stages. After fin ray development, at least some of the older larvae in the bay utilized vertical migration in relation to tidal flow to reenter the river. Those within the river demonstrated a similar behavior to remain there.

Post-larval Stage

Abundance

Post-larval winter flounder were collected using a 1-m beam trawl from late May through late September at stations LR and WA in the Niantic River. The standardized catch per 100 m^2 at both stations peaked on June 13 and began to decline thereafter, most likely after recruitment began to be offset by mortality, and stablized by late July (Fig. 14). Although densities were more variable at WA, juveniles were more abundant there during late summer (ca. 8 per 100 m^2) than at LR (ca. 6 per 100 m^2). This was also found in 1984 (NUSCo 1985).

A comparison of catches made from 1983 through 1985 at LR showed that although densities in 1984 were initially higher, they were similar in magnitude to 1985 by July (Fig. 15). The first 5 weeks of 1983 are not shown because the catch during that period was biased (NUSCo 1984), but based on abundance later in the year, juveniles were probably more numerous than during the other years. Although 1983 densities were within the range of those found for 1984 and 1985 during early July, abundance in late summer was greater. More variability was evident for 1983 as only three replicate tows were taken per sampling trip rather than the four made during 1983 and 1984.

difference agreed well with the 17-day difference in developmental time to Stage 4 between the two years (Table 9). The estimated time from hatching to Stage 4 in 1983 and 1984 was 56 and 73 days, respectively. This was longer than the estimated 49 and 63 days needed for growth from 3 mm to 7.5 mm in 1983 and 1984, respectively.

Although day 1 was defined at a length of 3 mm, it did not necessarily represent the date of hatching because little growth was expected during yolk-sac absorption. Cetta and Capuzzo (1982) reported that in a laboratory study total body weight of winter flounder larvae decreased during the first 2.5 weeks after hatching and after 1 week, larvae appeared to be metabolizing body tissue following depletion of the yolk sac. Therefore, an additional 10 days may pass during yolk-sac absorption when larvae are in the 3-mm size-class. Based on the growth curves, these additional days would increase the age of a 7.5-mm larva to 59 days in 1983 and 73 days in 1984, which agreed with the estimated developmental time to reach Stage 4 of 56 days in 1983 and 73 days in 1984.

Tidal Import and Export

Sampling was conducted at the Niantic River Highway Bridge on April 5 and May 8 during two tidal cycles to estimate the tidal import and export of winter flounder. On April 5, mostly Stage 1 and 2 larvae were collected and on May 8 the larvae were primarily Stage 3 (Fig. 16). More Stage 1 larvae were collected during ebb rather than flood tide in April, although about 40% (1,521 per 500 m^3) of the former were collected in one sample taken near the bottom. Slightly fewer Stage 2 and 3 larvae were collected during flood tide. On May 8, similar numbers of Stage 3 larvae were collected on the two tides and most of the Stage 4 larvae were collected during the flood.

Net tidal flushing for the predominant developmental stages on each sampling date was estimated by multiplying the density (average of mid and bottom samples) by the estimated water velocity during the time of sample collection. This density adjustment accounted for changes in discharge volume during the tidal cycle. A harmonic regression equation using a 12-h tidal cycle was fit to densities adjusted for
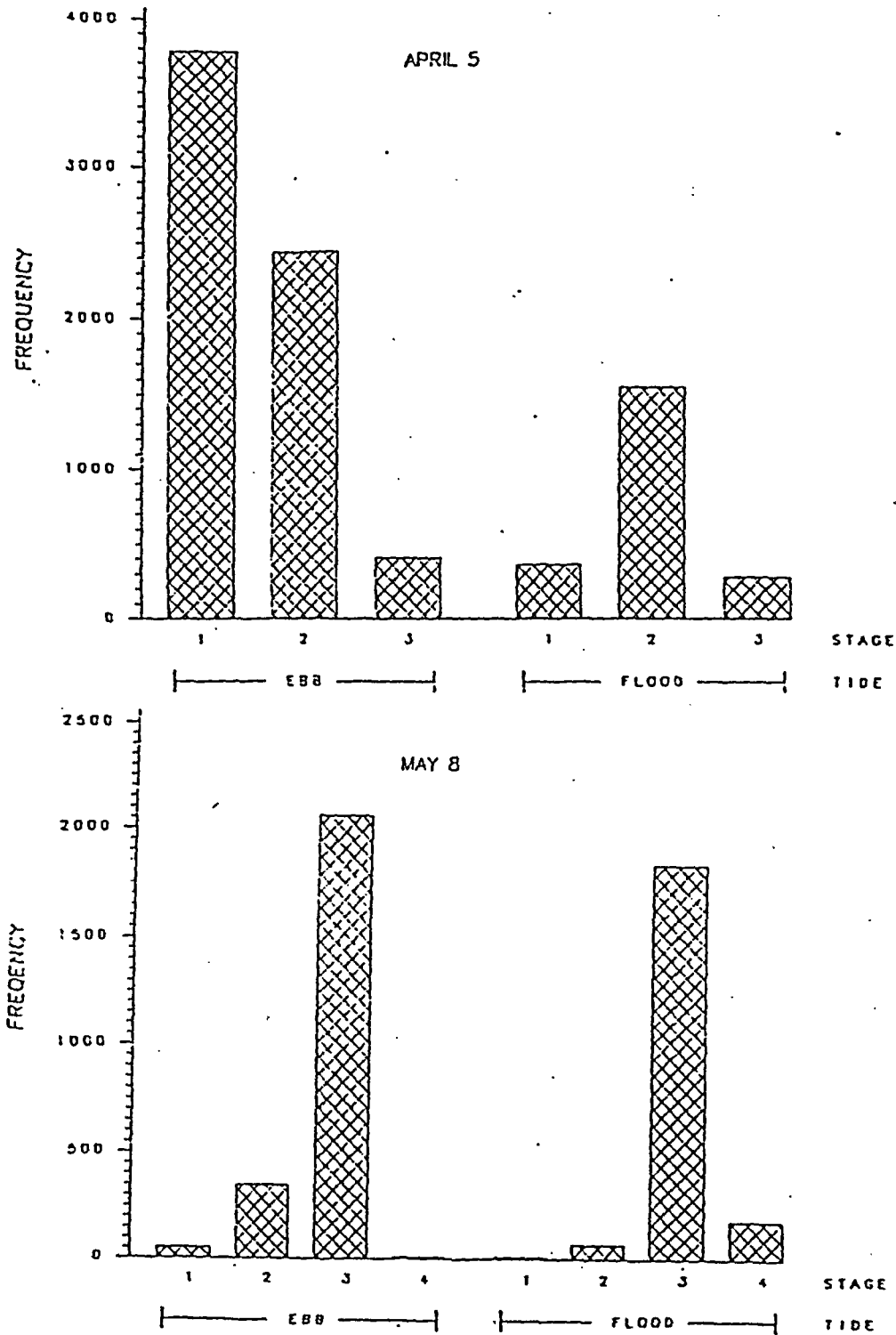
Results

Figure 16. Frequency of larval winter flounder by developmental stage collected at the mouth of the Niantic River during ebb and flood tides on April 5 and May 8, 1984.

velocity. Good fits were obtained for Stage 2 and 3 larvae on April 5 and for Stage 3 larvae on May 8 (Fig. 17). Densities of Stage 1 larvae on April 5 could not be modeled ($R^2=0.38$) because larvae were scarce during flood tide and the one sample with a very high density was collected during ebb tide. The area under the curve for each tidal stage was assumed to be a good estimate of net flushing and the areas were approximated by numerical integration of the respective harmonic regression equations using 5-min increments. On April 5, an estimated 65 and 63% of the Stage 2 and 3 larvae, respectively, returned to the Niantic River on a flood tide. The return of a flood tide increased to 92% for Stage 3 larvae on May 8. The larval dispersion model developed for winter flounder larvae in the Niantic River used a 72% return of passive particles to the river on a flood tide (Saila 1976). This model underestimated larval retention because older larvae used flood currents to increase their return to the river.

The data for May 8 showed a net loss of Stage 3 larvae from the river during a tidal cycle. This did not agree with the tidal import-export studies conducted in 1983, when many more larvae were found entering the river than leaving (NUSCo 1984). In 1983, sampling was conducted on May 9 and 18 and Stage 3 (45%) and 4 (48%) larvae predominated. Although they May 8 sampling in 1984 was conducted during a similar time period, larval development was slower this year (Table 9). Because of slower development in 1984, few Stage 4 larvae were present during the early part of May and the younger Stage 3 larvae may not have had fully developed fins. This lack of complete fin development possibly prevented the larvae from using tidal currents to enter the river as it was postulated in 1983.

24-h Tidal Studies

Two 24-h tidal studies were conducted in 1984 to examine the effect of tide on the observed abundance of early developmental stages (Stages 1 and 2). These studies were used to most efficiently schedule sampling for winter flounder larvae of various developmental stages. The 1983 24-h sampling was conducted when Stage 3 and 4 larvae predominated and

APRIL 5 STAGE 2
·65 % RETURN

$$\text{Exch} = -1968 \div 1540 \cos(h) + 14174 \sin(h)$$
$$R^2 = 0.91$$

APRIL 5 STAGE 3
63 % RETURN

$$\text{Exch} = -424 + 336 \cos(h) \div 2910 \sin(h)$$
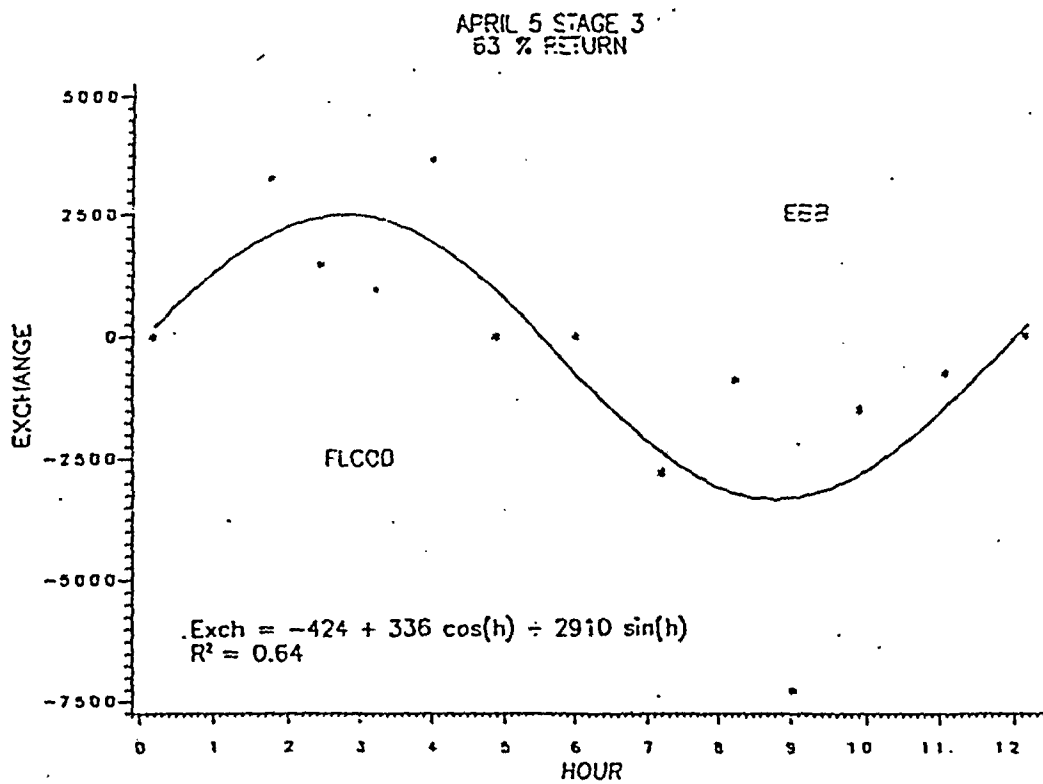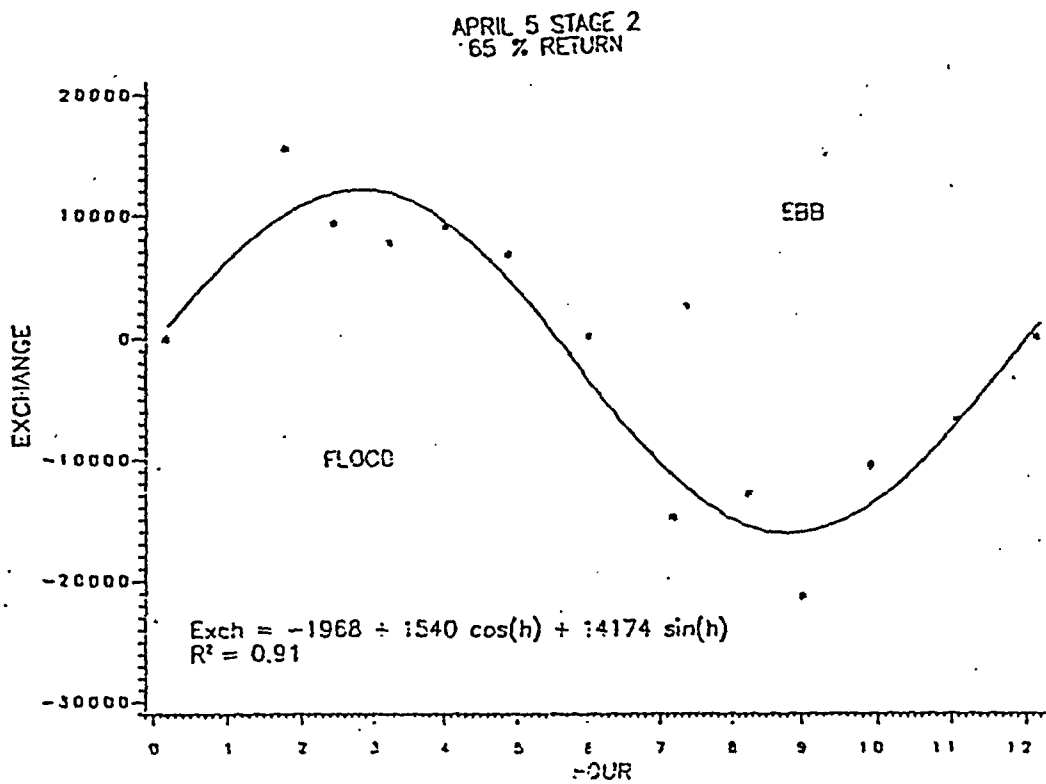$$R^2 = 0.64$$

Figure 17.   Estimated exchange of larval winter flounder at the mouth of the
Niantic River for Stage 2 and 3 larvae on April 5 and Stage 3
larvae on May 8 with the line fitted from a harmonic regression
model.

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MAY 8 STAGE 3
92 % RETURN

Exch = −430 + 3289 cos(h) − 14328 sin(h)
R² = 0.74

HOUR .
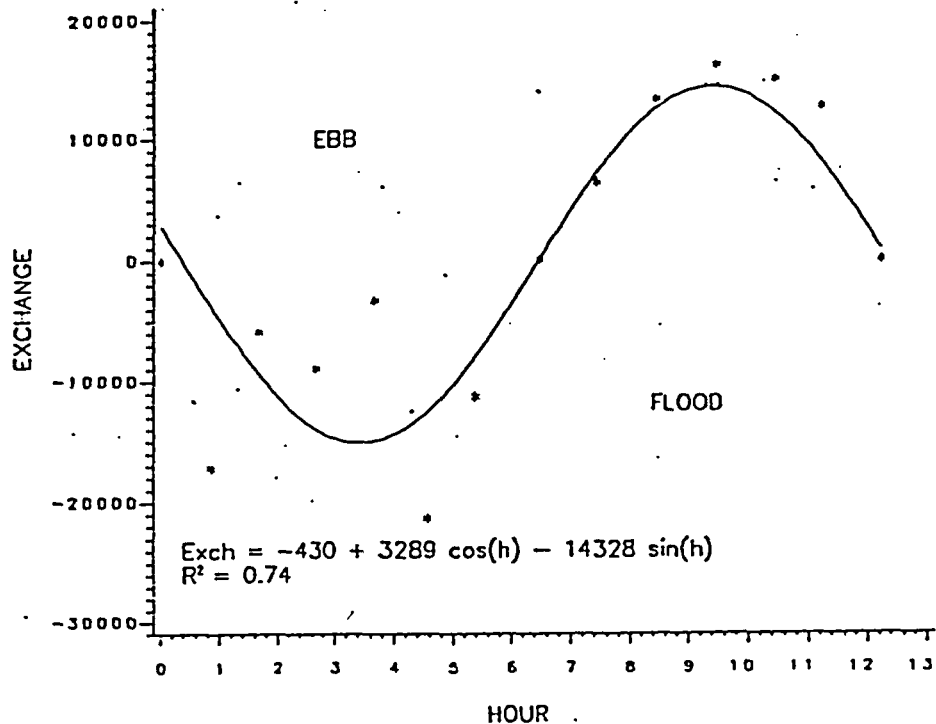
Figure 17. (Cont'd)

their collection densities increased during flood tides. This was attributed to a vertical movement in relation to tidal currents, which served as an estuarine retention mechanism.

In 1984, densities of Stage 1 and 2 larvae on March 12 were unrelated to tide (Fig. 18). Although the greatest density (746 per 500 m³) was found during one of three low slack tidal stages sampled, the remaining collection densities ranged from approximately 100 to 300. Collection densities evidently changed in relation to tidal stage on March 19. The greatest densities were found during an ebb tide and the lowest during a flood tide. Also, collection densities were smaller on March 12 than on March 19, as the largest ones from the first study were smaller than almost all those found a week later. A harmonic regression with a 12-h period was used in an attempt to relate changes in density to tidal stage. A satisfactory fit was achieved for Stage 1 larvae (R²=0.58), but not for Stage 2 (R²=0.01) (Fig. 19).

The increased abundance of Stage 1 larvae found during an ebb tide on March 19 agreed with the results of the previously presented tidal import-export study on April 4, when few Stage 1 larvae returned to the
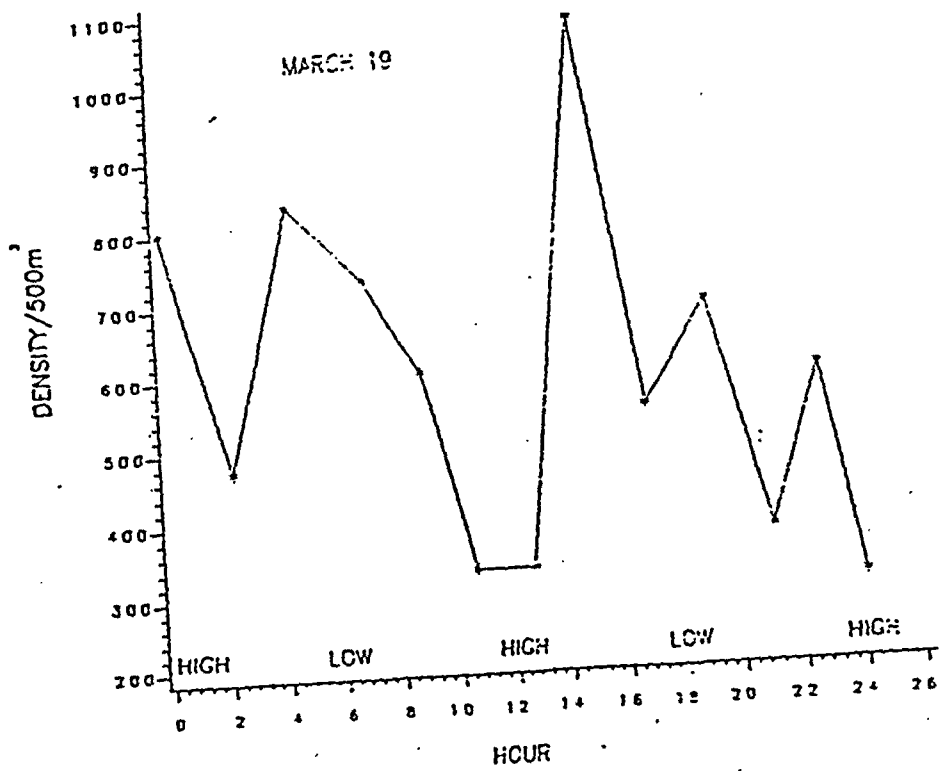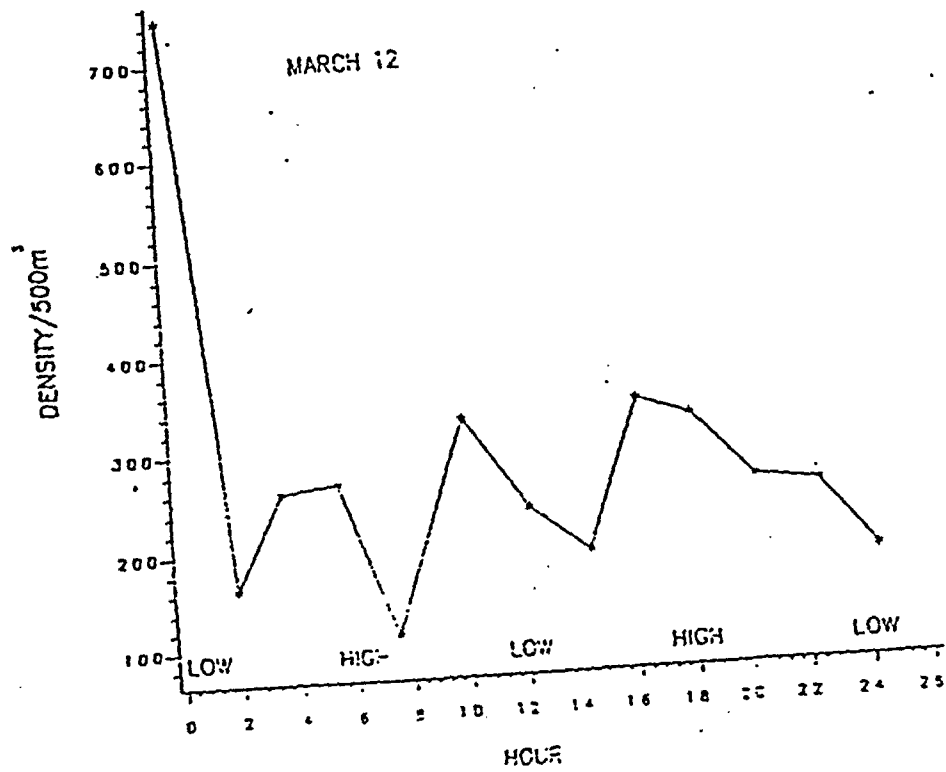
Figure 18. Larval winter flounder density per 500 m³ and the time of high and low slack tidal currents during the March 12 and 19, 1984 24-h studies at station C.
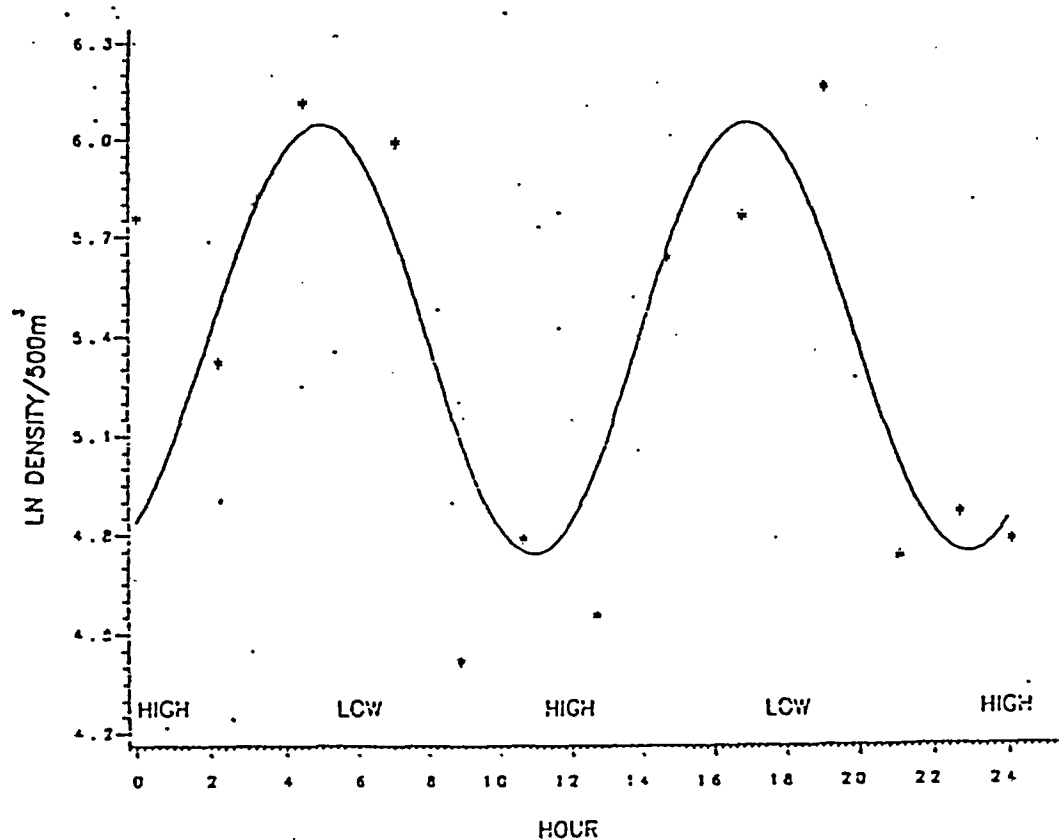
Figure 19. Stage 1 larval winter flounder abundance (ln density per 500 m³) and the time of high and low slack tidal currents for the March 19, 1984 24-h study at station C with the line fitted from a harmonic regression (ln density = $5.39 - 0.55 \cos(h) + 0.34 \sin(h)$; $R^2 = 0.58$).

Niantic River on a flood tide (Fig. 16). Although the same tidal flushing study indicated a net loss of Stage 2 larvae, apparently the difference in density between ebb and flood tides at station C was not sufficient to have been detected using the harmonic regression model.

Post-larval Stage
Abundance

A 1-m beam trawl was used at stations LR and CO in the Niantic River during 1984 to sample for young-of-the-year winter flounder following larval metamorphosis. As in 1983, dense mats of the alga _Enteromorpha clathrata_ developed at CO and hampered sampling there beginning in mid-July. Much algae collected on the tickler chain and

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NORTHEAST UTILITIES

THE CONNECTICUT LIGHT AND POWER COMPANY
WESTERN MASSACHUSETTS ELECTRIC COMPANY
HOLYOKE WATER POWER COMPANY
NORTHEAST UTILITIES SERVICE COMPANY
NORTHEAST NUCLEAR ENERGY COMPANY

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October 30, 1990

D04143

Ms. Leslie Carothers, Commissioner
Department of Environmental Protection
State Office Building
165 Capitol Avenue
Hartford, CT 06115

Dear Commissioner Carothers:

Millstone Nuclear Power Station
Ecological Monitoring Program

Northeast Utilities Service Company (NUSCO), as agent for Northeast Nuclear Energy Company (NNECO), agreed with Connecticut Department of Environmental Protection, Bureau of Water Management personnel in a meeting at Northeast Utilities Environmental Laboratory on September 11, 1990, to provide a report on or before October 31, 1990, describing winter flounder abundance indices for 1990; new methods for assessing larval entrainment effects, and a progress report on the fish return sluiceway systems at NNECO's Millstone Nuclear Power Station (MNPS). Accordingly, NUSCO hereby submits, on behalf of NNECO, updates of work completed in 1990 as follows:

o   1990 Abundance Indices for Winter Flounder (Enclosure 1)

o   Mass-Balance Calculations for Assessing Production Losses due to Entrainment of Winter Flounder (Enclosure 2)

o   Progress Report on the MNPS Fish Return Systems (Enclosure 3)

If you have any questions after reviewing this submission, please call Dr. William C. Renfro, Director, NUSCO Environmental Programs, at (203) 665-4620.

Very truly yours,

NORTHEAST UTILITIES SERVICE COMPANY
As Agent for Northeast Nuclear
Energy Company

E. J. Mroczka
Senior Vice President

Enclosures
cc: See next page

OS3422 REV. 4-88

MASS-BALANCE CALCULATIONS FOR ASSESSING PRODUCTION LOSSES DUE TO ENTRAINMENT OF WINTER FLOUNDER

MILLSTONE NUCLEAR POWER STATION
NORTHEAST NUCLEAR ENERGY COMPANY
NPDES PERMIT No. CT000326

BACKGROUND

Recent assessments of the potential impact of entrainment by Millstone Nuclear Power Station (MNPS) on the Niantic River winter flounder stock have assumed either an entrainment loss of a postulated percentage of the larvae produced in the River (NUSCO 1989, 1990) or that all winter flounder larvae entrained came from the Niantic River (Crecco and Howell 1990). Results from special studies conducted in 1988 suggested that many larvae entering Niantic Bay may not have come from the Niantic River stock. These studies consisted of 24-h sampling conducted in Twotree Island Channel and import/export sampling at the mouth of the Niantic River (NUSCO 1989). The geometric mean density of winter flounder larvae collected during a flood tide (from low to high slack current, 16 samples) during two 24-h sampling periods (April 25 and May 4, 1988) in Twotree Island Channel was 128 per 500 m³. These larvae would pass Millstone Point and potentially enter Niantic Bay. The average volume of water passing between Millstone Point and bell buoy 4 (about 0.5 nautical miles SW of Millstone Point) is approximately 2,200 m³/sec (NUSCO 1983). Therefore, about 13 million larvae should have entered Niantic Bay through this area during a flood tide. During a similar time period, two import/export studies (April 18 and May 2, 1988) were conducted at the mouth of the Niantic River and the geometric mean density of samples collected during an ebb tide (10 samples) was 155 per 500 m³. With an average tidal prism volume for the Niantic River of about 2.7 x 10⁶ m³ (Kollmeyer 1972), less than 0.9 million larvae left the river during an ebb tide. This number of larvae is equivalent to less than 7% of the larvae entering Niantic Bay between Millstone Point and bell buoy 4 during a flood tide. Therefore, at least for this period during 1988, a majority of the winter flounder larvae entering Niantic Bay and potentially entrained came from a source(s) other than the Niantic River.

MATERIALS AND METHODS

Mass-balance calculations were used to investigate whether the number of winter flounder larvae entering the Niantic Bay from the Niantic River could support the number of larvae observed in the Bay during the winter flounder larval season each year. There are three potential larval inputs to Niantic Bay: eggs hatching in the Bay, larvae flushed from the Niantic River, and larvae entering the Bay across the boundary between Millstone Point and Black Point. Due to the low numbers of yolk-sac larvae collected in Niantic Bay (NUSCO 1985-90), minimal spawning and subsequent hatching is thought to occur in Niantic Bay and, therefore, would be a negligible larval source. It is known that larvae are flushed from the Niantic River to the Bay and the number entering can be estimated from available data. Although it was demonstrated above that a large number of larvae entered Niantic Bay from Long Island Sound (LIS) in 1988 during at least a portion of the larval season, insufficient data were available to quantify this source throughout the entire larval season and for different years. Therefore, this source remains a potentially important one that needs further investigation. There are four potential losses of larvae from Niantic Bay: larvae enter the Niantic River during a flood tide, are lost due to natural mortality, entrained by MNPS, and larvae are flushed from the Bay into LIS. The number entering the Niantic River can be estimated from available data, and estimates of natural mortality and entrainment have been made, but little is known about the number of larvae flushed to LIS and this remained an unknown in the following calculation. The form of the mass balance equation was:

$$NB_{t+5} = (NB_t) - (Ent) - (Mort) + (FromNR) - (ToNR) \pm (Source/Sink)$$

where t = time in days

NB_{t+5} = number of larvae in Niantic Bay 5 days after day t (instantaneous daily estimate)

NB_t = initial number of larvae in Niantic Bay on day t (instantaneous daily estimate)

Ent = number of larvae lost from Niantic Bay due to entrainment in the condenser cooling water system (over a 5-day period)

Mort = number of larvae lost from Niantic Bay due to natural mortality (over a 5-day period)

FromNR = number of larvae flushed from the Niantic River (over a 5-day period)

ToNR = number of larvae entering the Niantic River (over a 5-day period)

Source/Sink = unknown number of larvae in Niantic Bay that flush to LIS or enter the Bay from LIS (over a 5-day period)

Solving for the unknown Source/Sink term, the equation was rearranged as:

$$\text{Source/Sink} = (NB_{t+5}) - (NB_t) + (Ent) + (Mort) - (FromNR) + (ToNR)$$

and because these mass-balance calculations were based on the change in the number of larvae in Niantic Bay over a 5-day period:

$$\text{5-day Change} = (NB_{t+5}) - (NB_t)$$

therefore:

$$\text{Source/Sink} = (\text{5-day Change}) + (Ent) + (Mort) - (FromNR) + (ToNR)$$

The selection of 5 days as the period of change was arbitrary and the use of different time periods should not alter the conclusions from the mass-balance calculations. Daily abundance estimates were derived from the Gompertz equation. The equation was fitted to weekly geometric mean densities (NUSCO 1990). Daily estimates for Niantic Bay (NB_t and NB_{t+5}) were calculated from data collected at stations NB and EN combined, which represented an instantaneous daily standing stock after adjusting for the volume of Niantic Bay (about 50×10^6 m³; E. Adams, MIT, personal communications). The difference between these two estimates was the term (5-day Change). Daily entrainment estimates were based on data collected at station EN and the actual daily volume of condenser cooling water used at MNPS. The daily entrainment estimates were summed over each 5-day period (Ent). Stage-specific mortality rates were determined by Crecco and Howell (1990) and modified to daily stage-specific mortality rates by assuming stage durations of 10 days for Stages 1, 3, and 4 larvae; and 20 days for Stage 2 larvae. The proportion of each stage collected at station EN during each 5-day period was applied to the daily standing stock for Niantic Bay (NB_t) to estimate the number of larvae in each developmental stage for stage-specific mortality calculations. The daily loss due to natural mortality was summed for each 5-day period (Mort).

The 5-day input of larvae to Niantic Bay from the River (FromNR) was based on daily density estimates for station C in the river after adjusting for the rate of flushing between station C and the mouth the of River (Fig. 1). To estimate the relationship between the estimated daily density at station C and the average density of larvae leaving the River on an ebb tide, the geometric mean density of samples collected during an ebb tide for 10 import/export studies conducted at the mouth of the Niantic River during 1984,1985, and 1988 (NUSCO 1985, 1986, 1989) were compared to the estimated daily densities at station C. It appeared that less than 50% of the larvae estimated to be at station C were flushed from the river. Therefore the average density of larvae flushed from the Niantic River was estimated by the regression equation:

$$\text{Average density} = 18.828 + 0.458 \,(\text{Daily density at station C})$$

This average density, the average tidal prism of 2.7×10^6 m³ (Kollmeyer 1972), and about 1.9 tidal prisms per day were used to estimate the daily flushing of larvae from the River to Niantic Bay. This daily input to the Bay was summed for each 5-day period to calculate the term (FromNR) in the mass-balance equation. The loss of larvae from Niantic Bay to the River during a flood tide (ToNR) was based on the daily density estimates for Niantic Bay (stations NB and EN

combined). A comparison of the daily estimated density for Niantic Bay to the geometric mean density of the samples collected during a flood tide for the 10 import/export studies indicated no significant relationship (Fig. 2). Because there was no apparent systematic bias in this relationship and for lack of better information, the estimated daily densities for Niantic Bay from the Gompertz equation were used to estimate daily loss after adjusting for the average tidal prism and the number of tidal prisms per day. These daily estimates of the number of larvae entering the river during a flood tide were summed over each 5-day period to calculate the term (ToNR) in the mass-balance equation.

The Source/Sink term represents the net loss from or gain to Niantic Bay of larvae from LIS during a 5-day period that is required to balance the calculation. For a net loss this term would be negative and for a net gain the term would be positive.

RESULTS AND DISCUSSION

Mass-balance calculations were made for 1984 through 1989 with four of these years (1986-89) during 3-unit operation. The results of calculations for each 5-day period in 1989 are provided as an example (Table 1). During the season the value of the term (5-day Change) went from positive to negative when the estimated number of larvae in Niantic Bay started to decline during a 5-day period. In the first part of the larval season there was a net loss of larvae from Niantic Bay (negative Source/Sink term). Starting in early April the Source/Sink term became positive, indicating that larvae from other sources (LIS) were required to support the change in larval abundance or to balance the equation. During peak entrainment (mid-April), more larvae were imported from LIS than were entrained suggesting that this was an important larval source for Niantic Bay. For each 5-day period the proportion of entrainment attributed to the Niantic River was estimated from the ratio of larvae entering the Bay from the River (FromNR) to the total input from both sources (FromNR + Source/Sink). This proportion was applied to the total number entrained to estimate the number entrained from the Niantic River. For the 5-day periods when there was a net loss (negative Source/Sink term) or when the proportion from the river was greater than one, all larvae entrained were assumed to have originated from the Niantic River. Estimates of annual total entrainment and the annual number entrained from the Niantic River were determined by summing all 5-day periods. Based on mass-balance calculations for data collected in 1984-89, 28.2 to 65.7% of winter flounder larvae entrained by MNPS originated from the Niantic River (Table 2). Except for 1984, the total entrainment estimates based on the daily densities derived from the Gompertz function were 19 to 39% larger than those previously reported (NUSCO 1990), which were calculated from median entrainment densities. The reason for this increase was not immediately evident, but these larger annual entrainment estimates were used for the remaining calculations.

The number of each developmental stage entrained during each 5-day period was estimated based on the proportion of each stage collected at station EN during the period. By applying the proportion entrained attributed to the Niantic River (FromNR / Σ of FromNR and Source/Sink) the number of larvae in each stage was allocated to the two sources for each 5-day period. The annual number of larvae entrained by stage from each source was estimated by summing all 5-day periods (Fig. 3). Except for 1984, most of the Stage 3 larvae (the predominant stage entrained) originated from sources other than the Niantic River. The estimated number of larvae entrained by stage from the River was compared to the annual abundance estimates for each larval stage in the Niantic River (Crecco and Howell 1990). The estimated percentage of the Niantic River winter flounder production that was entrained annually since 1984 ranged from about 6 to 17% (Table 3). These estimates of year-class strength reductions can be used in further impact assessment work using the stochastic population model.

CONCLUSION

Mass-balance calculations were used, as an empirical analysis on available data, to estimate the proportion of the Niantic River production that was annually entrained. The results indicated that a large number of entrained larvae were from a source or sources other than the Niantic River. A special sampling program is presently being designed for the 1991 larval winter flounder season to help verify these results.

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Table 1. Results of mass-balance calculations for each 5-day period in 1989.

Start of 5-day Period	5-day Change (x 10^6)	Number Entrained (x 10^6)	Loss due to Mortality (x 10^6)	Number from Niantic R. (x 10^6)	Number to Niantic R. (x 10^6)	Source/Sink Term (x 10^6)
2-15	0.0[a]	0.0[a]	0.0[a]	1.8	0.0[a]	-1.8
2-20	0.0	0.0	0.0	9.2	0.0	-9.2
2-25	0.0	0.0	0.0	31.4	0.0	-31.4
3-02	0.0	0.0	0.0	62.0	0.0	-62.0
3-07	0.0	0.0	0.0	82.8	0.0	-82.8
3-12	0.0	0.0	0.0	85.3	0.0	-85.2
3-17	0.3	0.0	0.0	74.2	0.1	73.7
3-22	1.4	0.3	0.3	57.9	0.6	-55.3
3-27	3.7	2.7	1.2	42.2	2.1	-32.6
4-01	5.8	11.1	3.7	29.5	5.0	-3.8
4-06	6.1	21.4	4.3	20.1	8.7	20.4
4-11	4.3	21.5	7.1	13.6	11.9	31.2
4-16	1.6	26.0	7.4	9.2	13.7	39.6
4-21	-0.9	24.1	6.3	6.3	13.9	37.2
4-26	-2.5	19.6	6.3	4.3	12.8	31.9
5-01	-3.2	17.2	5.3	3.1	11.1	27.2
5-06	-3.2	12.3	4.0	2.3	9.1	19.9
5-11	-2.9	7.4	3.0	1.8	7.2	12.9
5-16	-2.5	3.5	2.3	1.5	5.6	7.4
5-21	-2.0	2.3	1.8	1.3	4.2	5.1
5-26	-1.5	1.8	1.3	1.1	3.2	3.6
5-31	-1.2	1.2	1.0	1.1	2.4	2.3
6-05	-0.9	1.0	0.7	1.0	1.7	1.5
6-10	-0.7	0.6	0.5	1.0	1.3	0.8
6-15	-0.5	0.4	0.0	0.9	0.9	-0.1
6-20	-0.4	0.3	0.0	0.9	0.7	-0.3

[a] Due to rounding zero values represent numbers under 50,000 larvae.

Table 2. Larval winter flounder estimates of total entrainment, number of larvae entrained from the Niantic River, and the percentage of total entrainment attributed to the Niantic River for 1984-89 based on mass-balance calculations.

Year	Total Entrainment (X 10^6)	Niantic River Larval Entrainment (X 10^6)	% Entrainment Attributed to the Niantic River
1984	87.8	57.7	65.7
1985	82.6	43.2	52.3
1986	130.1	51.4	39.5
1987	171.8	66.8	38.9
1988	192.0	60.8	31.7
1989	174.8	49.3	28.2

Table 3. Estimated abundance of winter flounder larvae in the Niantic River and the number and percentage of the production entrained from the Niantic River by developmental stage for 1984-89. The number of larvae from the Niantic River was based on mass-balance calculations.

Developmental Stage	Niantic River[a] Abundance (x 10^6)	Entrainment from Niantic River (x 10^6)	% of the Production
		1984	
Stage 1	3096	0.3	<0.1
Stage 2	743	24.8	3.3
Stage 3	364	26.5	7.3
Stage 4	255	6.1	2.4
Total		57.7[b]	13.0
		1985	
Stage 1	4071	4.0	0.1
Stage 2	977	22.4	2.3
Stage 3	479	14.3	3.0
Stage 4	335	1.2	0.3
Total		41.9	5.7
		1986	
Stage 1	2696	1.2	<0.1
Stage 2	755	11.9	1.6
Stage 3	392	25.9	6.6
Stage 4	275	8.4	3.1
Total		47.4	11.3
		1987	
Stage 1	3281	1.1	<0.1
Stage 2	919	21.4	2.3
Stage 3	478	38.5	8.1
Stage 4	334	4.4	1.3
Total		65.4	11.7
		1988	
Stage 1	5352	4.7	0.1
Stage 2	803	12.5	1.6
Stage 3	289	39.0	13.5
Stage 4	208	3.1	1.5
Total		59.3	16.7
		1989	
Stage 1	4421	3.6	0.1
Stage 2	619	15.6	2.5
Stage 3	204	27.6	13.5
Stage 4	137	1.7	1.2
Total		48.5	17.3

[a]Abundance estimates are from Creeco and Howell (1990).

[b]Some total entrainment estimates may differ slightly from Table 2 due to rounding error.

Fig. 1. Comparison of winter flounder larval densities collected during an ebb tide at the mouth of the Niantic River and corresponding daily density estimates at station C in the Niantic River from the Gompertz function.

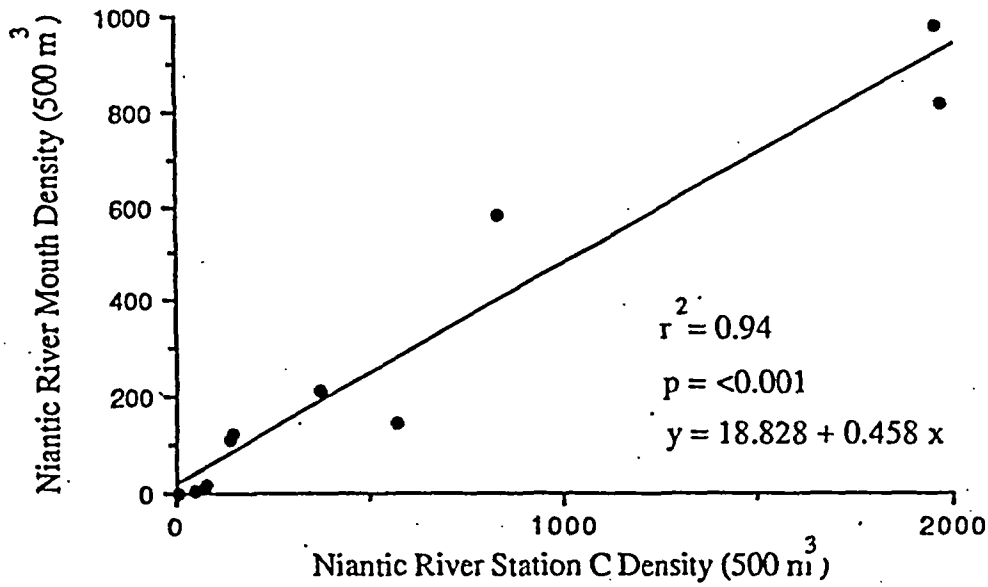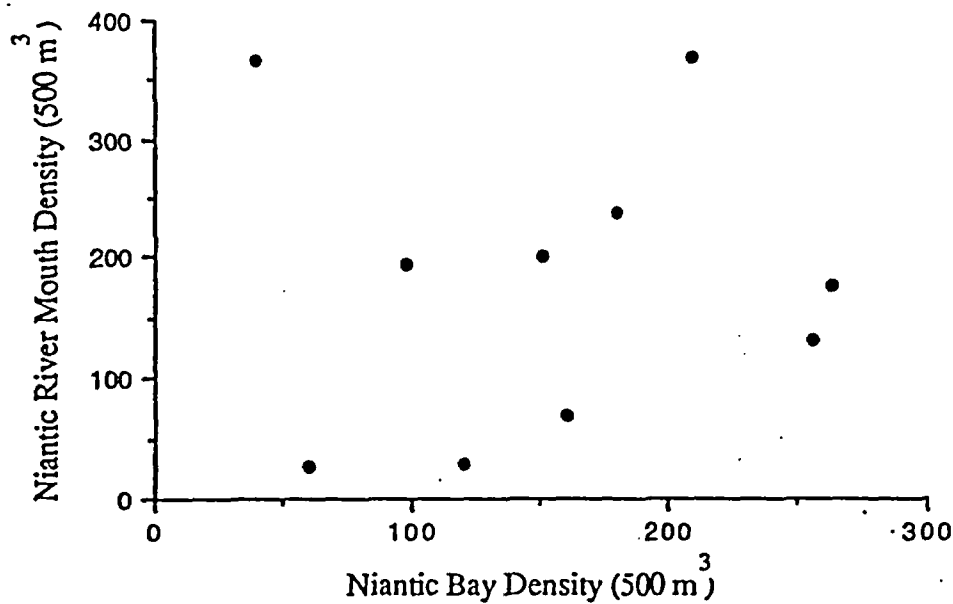Fig. 2. Comparison of winter flounder larval densities collected during a flood tide at the mouth of the Niantic River and corresponding daily density estimates in Niantic Bay from the Gompertz function.
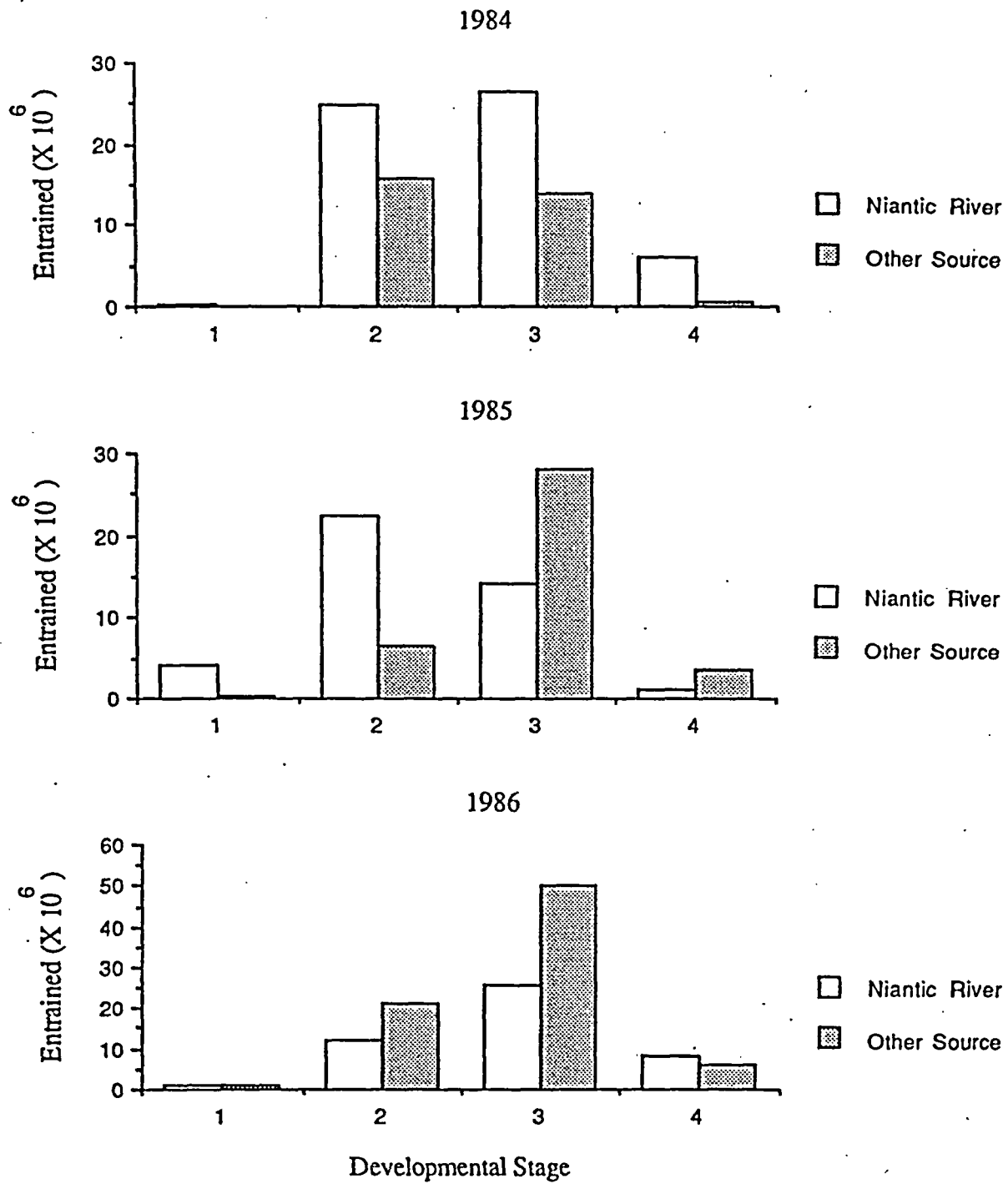
Fig. 3. Estimated number of winter flounder larvae entrained by developmental stage from the Niantic River and other source(s) based on mass-balance calculations.

1987

1988

1989
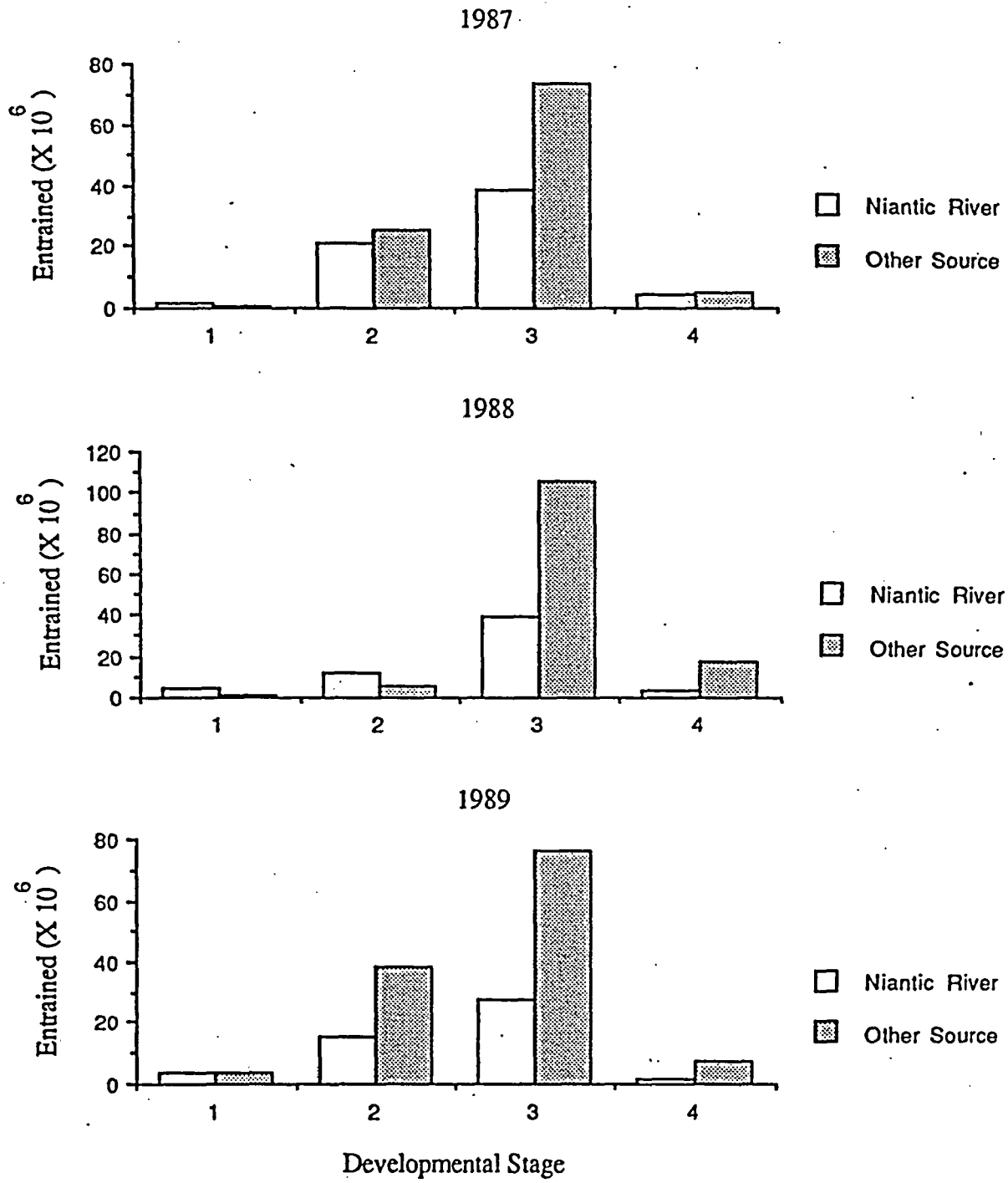
Fig. 3. Cont'd.

<u>Report to Millstone Environmental Laboratory, Ecological Advisory Committee</u>
<u>Analysis of winter flounder Larvae</u>
<u>By Dr. J. Crivello, University of Connecticut, Storrs, CT</u>
<u>2.12.02</u>

This is a report of the activities of the second year of a project designed to determine the most likely source population for winter flounder larvae entrained by the Millstone Power Station. Staff scientists at the Environmental Laboratory, Millstone Power Station, Waterford, CT, provided samples. Larvae were collected from the Niantic River, Thames River and an area due west of the Connecticut River, mostly off Westbrook (Figure 1). These areas were known to be, or adjacent to winter flounder nursery locations in Long Island Sound (LIS), and were near the Millstone Station. Larvae samples were staged according to criteria presented in NUSCO (2000). Stage 1 (yolk-sac) & 2 (pre-flexion) winter flounder larvae were collected with a bongo net sampler with a 202-μm mesh net at variable depths from February through April 2001. Larvae were sorted on board the sampling vessel and placed in 70% ethanol. A total of 164 Stage 1 & 2 larvae were collected from the Niantic River, 174 from the Thames River area and 198 from the Westbrook area (Table 1).
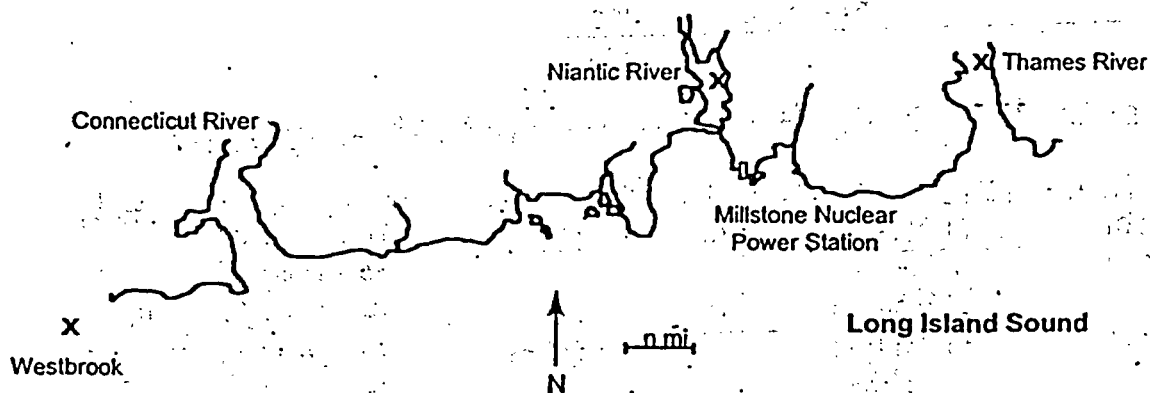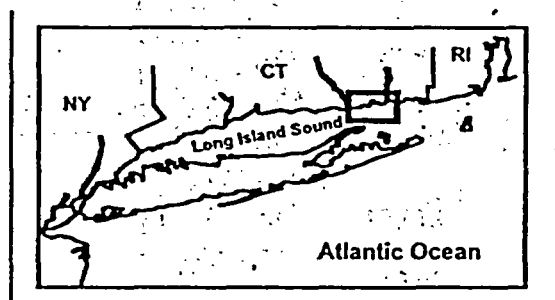
Figure 1. Approximate locations in eastern Long Island Sound (X) where larval winter flounder were collected for genetic stock identification studies during 2001.

Larvae were also collected, using a 333-micron mesh net, from seawater entrained at the Millstone Power Station from March through June 2001. Larval samples collected during this time period were stratified so as to provide an accurate cross-section of larvae entrained by the Station

(i.e., the maximal number of sample were collected during those dates when it was known that the maximal number of larvae are entrained in the Station). A total of 1067 stage 2, stage 3 (flexion) and stage 4 (pre-metamorphosing) entrainment larvae were collected (Table 2).

Table 1. Spawning Stock collection sites and number of larvae processed

Collection site	Date	Number and stage	Total
Niantic River	2.27.01	35 - stage 1	
		25 - stage 2	
	3.14.01	31 - stage 1	
		19 - stage 2	
	3.28.01	19 - stage 1	
		35 - stage 2	164
Thames River	3.03.01	12 - stage 1	
		44 - stage 2	
	3.11.01	19 - stage 1	
		42 - stage 2	
	3.18.01	12 - stage 1	
		45 - stage 2	174
Westbrook	4.05.01	30 - stage 1	
		50 - stage 2	
	4.16.01	24 - stage 1	
		34 - stage 2	
	4.26.01	6 - stage 1	
		3 - stage 2	
		51 - stage 3	198
			536

Table 2. Entrained larvae collection dates & amounts and number of larvae processed

Collection Dates	Number and stage	Total	Collection Dates	Number and stage	Total
3.28.01	14 - stage 2	14	5.14.01	28 - stage 2	
4.06.01	4 - stage 2			66 - stage 3	
	7 - stage 3	11		10 - stage 4	104
4.09.01	45 - stage 2		5.21.01	43 - stage 3	
	34 - stage 3	79		20 - stage 4	63
4.16.01	47 - stage 2		5.29.01	41 - stage 3	
	95 - stage 3	142		10 - stage 4	51
4.23.01	14 - stage 2		6.04.01	15 - stage 3	
	176 - stage 3	190		19 - stage 4	34
4.30.01	18 - stage 2		6.11.01	29 - stage 4	29
	160 - stage 3	178	6.18.01	18 - stage 4	18
5.07.01	29 - stage 2				1067
	104 - stage 3				
	21 - stage 4	154			

Following metamorphosing and settlement, juvenile winter flounder (10-71 mm) were collected on June 22nd and September 18th of 2000 and July 3rd and September 24th of 2001 with a 1-meter beam trawl at two locations in the Niantic River (Figure 2: LR and WA). Juveniles were placed in 70% ethanol and then transferred to the lab for analysis. A small piece of muscle tissue was used to isolate genomic DNA from each juvenile (Table 3).
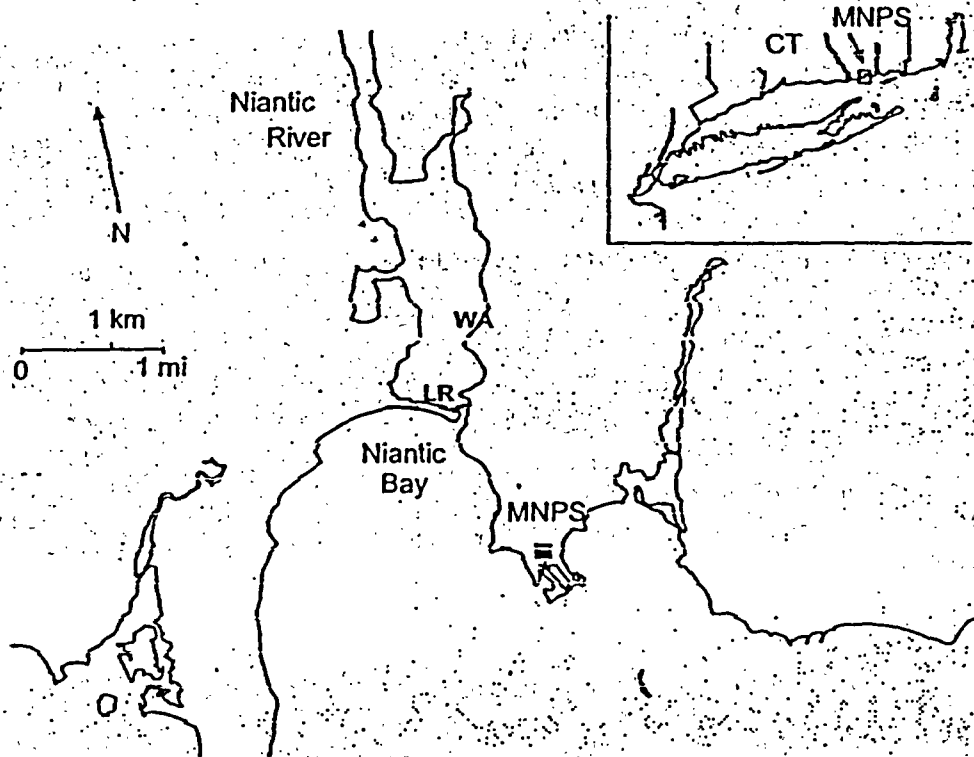
Figure 2. Approximate locations in the Niantic River (LR and WA) where age-0 juvenile winter flounder were collected for genetic stock identification studies during 2000 and 2001.

Table 3. Age-0 juvenile collection dates and number processed

Collection Site (Niantic River)	Date	Number	Collection Site (Niantic River)	Date	Number	Total
LR	6.22.00	57	WA	6.22.00	35	92
	9.18.00	30		9.18.00	35	65
	7.03.01	70		7.03.01	78	148
	9.24.01	45		9.24.01	18	63

Genetic analysis

Genomic DNA was extracted from each sample by the method of Kaplan *et al.* (2001) from each larva and juvenile muscle sample. Genomic DNA was quantified with Pico Green™ (Molecular Probes, Inc.) and comparison to a DNA standard curve. Stage 1 larvae provided 75-100ng of genomic DNA that was sufficient for analysis of 6 microsatellite loci. Stage 2 through 4 larvae and age-o juveniles gave large amounts of genomic DNA.

To analyze each microsatellite loci, 10ng of genomic DNA was added to a solution containing 10mM Tris, 50mM KCl, 2.5mM MgCl$_2$, 0.2mM dNTPs, 0.2µM forward and reverse primers to a final 10µl volume. The forward primer was covalently modified with a D2, D3, or D4 fluorescent tag (Research Genetics Inc. Huntsville, Alabama). The sequence of primers is included in Table 4 as well as the PCR conditions for each primer set. Primer sequences were a kind gift from Susan Douglas and Doug Cook (McGowan & Reith, 1999).

After PCR, the samples were precipitated by addition of 2µl 3M NaAcetate pH 5, 2µl of a 1mg/ml glycogen solution and 50µl of absolute ethanol. The samples were frozen at -70°C for ten minutes and then spun at 30,000xg for 15 minutes. The supernatant was discarded and each sample washed with 75µl of 70% ethanol. The samples were dried and re-suspended in 30µl of formamide that contained a 60-400 bp DNA standard labeled with a D1-fluorescent tag (Beckman Instruments, Pal Alto, CA). The samples were then analyzed on the Beckman Seq-2000™ Capillary Electrophoresis System (frag3 protocol). Microsatellite products were identified by size with an accuracy of 0.25 bp by comparison to standards. Table 4 contains the size ranges and number of alleles for each loci.

Statistical analysis

Statistical analyses of data were performed using PopGene (available as shareware at http://www.ualberta.ca/~fyeh/) and the NeuroShell™ Classifier neural net software (Ward Systems Inc, Frederick, MD). PopGene calculated expected heterozygosities as well as an estimate of F_{IS} (Cockerham and Weir, 1986). Tests for conformity to Hardy-Weinberg equilibrium were calculated using a Markov chain method. Tests for allele frequency differences were calculated using Fisher's exact test with pair-wise comparison of all samples at all loci that were then combined across loci. Genetic differences were also calculated.

The NeuroShell™ Classifier neural net software was used to assign entrained larvae to likely source locations. This software makes no assumptions about genetic differences among populations and builds an algorithm (i.e., a neural network) that best differentiates differences among the populations. This neural network is then applied to the entrained data. The network is trained on a file (i.e., the training file) that contains the genetic information about larvae collected from the three source areas.

Control experiments to determine the accuracy and resolving power of this approach were carried out in the following manner. A validation experiment was carried out in which the network was trained on one-half of the training sets and then used to classify the other half of each training set. This was repeated 100 times by randomly selecting which samples were included in the training set and which were classified. Through these experiments a mean error rate confidence value was generated. Then the network was trained on the complete training sets (TNN). In a second set of control experiments, the source location of each larva within the training groups was randomized (n=100). The randomized training sets were used to develop networks that were applied to entrained samples (RNN).

After the control experiments were carried out, the TNN was used to classify all entrained larvae and juveniles to the most likely geographical source. The TNN generated a confidence value for the classification of each unknown sample from 0 → 1.0. Samples were assigned to a geographical source population if confidence value exceeded 0.75. A confidence level of 0.75 was chosen for the following reasons: 1) this confidence level would be at least 3 times as great as the next high confidence value and 2) it represents the lowest confidence value with error less than 5% (determined by multiple classification of the same sample). In some cases, the TNN could not assign a sample to a source area with 0.75 confidence but was able to determine that the sample did not belong to a specific source population (less than 0.05 confidence). If the assigned confidence value was the same or similar for all three source populations the sample was assigned to an unknown group. This gave 7 possible groups: Niantic River, Thames River, Westbrook (Connecticut River), not-Niantic River, not-Thames River, not-Westbrook or an unknown location.

Results:

A major goal of this year's effort was to increase the number of identified alleles, to increase the number of larvae in the source populations and to make the collection of entrained larvae represent with the overall entrainment (i.e., the greatest number of analyzed entrained larvae should come from the date in which the greatest number of larvae are entrained by the plant)

These objectives were meet with an increase of 30% in the number of source population larvae (536 in 2001 vs. 423 in 2000). The number of entrained larvae also increased by 3-fold (1067 in 2001 vs. 360 in 2000) and the collection of larvae peaked during late April usually when the greatest numbers of larvae are entrained at the plant, although in 2001, entrained larvae were also abundant in May (DNC, in preparation). The number of identifiable alleles increased from 29 in 2000 to 135 alleles in 2001, thereby increasing the resolving power of the analysis.

Table 4. Primers and PCR conditions, microsatellite sizes, and number of alleles.

Loci	Primer Sequence	Product length (bp)	Annealing T°C	Allele Number
P157	D2-AGTGCAACAACAGATTCCAG(+) GCAGAATGAGTGAAATGTGG(-)	93-195	50°C	40
P159	D3-GTGTGGAGGTCAATGC(+) GGAGCATCATTCATACAC(-)	85-209	53°C	11
A441	D2-CAACTGTGGGTATGTGCCTG(+) GTGTCAGCACTGTGCTTAAACC(-)	89-213	55°C	25
D34	D4-GCCTGGTCTCATTGTGTTCC(+) AGGTTAAATGATTTCCTGAAGCTG(-)	89-315	55°C	27
I29	D3-GCTTCGGTTACACCTTTGC(+) AGGACAGTGAGGATGTCCG(-)	91-223	55°C	4
J42	D4-CACAAACTCAAGATGTTGCG(+) AAGCTCACTGGAAAATAATACCC(-)	95-185	55°C	28

D2, D3 & D4 refer to fluorescent tags on the forward primer.

Individual larvae could have 12 possible products (2 for each primer set) if each locus was heterozygous or one if the locus is homozygous (to a minimum of 6 products). The source populations were examined by POPGENE statistical package. In Table 5, the relevant characteristics of each microsatellite loci are provided. The p value refers to the likelihood that the loci obey Hardy-Weinberg rules (a p value >0.05 suggests that it does not). Loci p159 and I29 don't always obey Hard-Weinberg rules suggesting that they might be inbred but the F_{IS} values don't suggest that the loci are inbred. The Het_{obs} values refer to the heterozygosity of the loci among the tested samples. The heterozygosity varied from about 10-80%, which is typical for microsatellite loci. Five out of the 6 loci were very heterozygotic (thereby increasing their resolving power) while one (I29) was not very heterozygotic. In the future, it may be worthwhile to substitute a different marker than the I29 to increase resolving power. It is interesting to note that on the basis of the heterozygosities, the Thames River larvae were less heterozygotic than the Niantic or Westbrook. The Westbrook larvae were the most genetically diverse and may reflect more than one population as these larvae were collected in the open waters of LIS rather than within a specific estuary.

Table 5. Summary statistics for 6 microsatellite loci surveyed in winter flounder.

Population	Microsatellite Loci					
	P157	P159	A441	D34	I29	J42
Niantic						
P value	0.0000	0.2798	0.0000	0.0000	0.2759	0.0000
Het_{obs}	0.7481	0.3926	0.6000	0.7185	0.0667	0.5333
N	148	148	148	148	148	148
F_{IS}	0.1485	-0.0515	0.3151	0.1823	-0.0345	0.3597
Thames						
P value	0.0000	0.9817	0.0000	0.0000	0.0000	0.0000
Het_{obs}	0.8129	0.0719	0.5971	0.5972	0.1000	0.3669
N	154	154	154	154	154	154
F_{IS}	0.0937	-0.0281	0.3263	0.2985	0.1078	0.5927
Westbrook						
P value	0.0000	0.8865	0.0000	0.0000	0.1594	0.0000
Het_{obs}	0.7744	0.2359	0.6821	0.4974	0.0103	0.3538
N	195	195	195	195	195	195
F_{IS}	-0.0062	-0.0572	0.2133	0.4256	-0.0052	0.5716

P values indicate the probability of conformity to Hardy-Weinberg expectations by the Chi-squared method. N is the sample size.

The next comparison was to see how distinct the source populations were from each other. This is determined through the F_{ST} (Fisher's statistic), which is a numerical measurement of the genetic difference, with values greater than 0.05 considered to be significantly genetically distinct and values between 0.025 and 0.05 considered to show less significant but potentially important genetic differences. Table 6 has the results for 2000 & 2001. It is interesting to note that the Niantic River population is distinct from the Thames & Connecticut River area populations in both years. The Thames River larvae are also distinct from the Plum Bank and Westbrook larvae. This

genetic differentiation is geographically linked, i.e., those source populations that most geographically distinct are the most genetically distinct. The other interesting point is that the genetic differences were relatively similar over the last two years, suggesting a temporal stability.

Table 6. The genetic difference between training groups.

| | F_{ST} 2001 | | F_{ST} 2000 | |
Vs.	Thames	Westbrook	Thames	Plum Bank
Niantic	0.0385	0.0571	0.0384	0.0518
Thames	----	0.0545	----	0.0425
Westbrook		----		----

The NeuroShell neural network classifying program was then trained on the files containing the genetic differences between larvae collected in the Thames, Niantic and Connecticut Rivers. Control experiments were carried out in the following manner. Initially, the neural network was trained on half of the larva from each training area and then used to classify the other half of larva from the same area. Secondly, the order of the samples within the training set(s) was randomized but the correct source location was maintained. Thirdly, both the order and source of the larva in the training set was randomized. These controls demonstrated that this approach had at least 98.5% accuracy in classifying unknown larvae to one of the spawning areas. Samples were assigned to a geographical nursery population if their probability exceeded 0.75. A confidence level of 0.75 was chosen for the following reasons: 1) this confidence level would be at least 3 times as great as the next high confidence value and 2) it represents the lowest confidence value with error less than 5% (determined by multiple classification of the same samples with neural networks). In some cases, the network could not assign a sample to a nursery area but was able to determine that the sample did not belong to a specific nursery population (essentially less than 5% confidence). If the assignment confidence was the same for all 3-nursery populations the sample was assigned to an unknown group. This gave 7 possible groups: Niantic River, Thames River, Westbrook (Connecticut River), not-Niantic River, not-Thames River, not-Westbrook or an unknown location.

It is clear from Table 7 that there were very few larvae that could not be assigned to one of the 1st 6 groups (essentially not from any of the tested source areas). The greatest number of entrained larvae came from the Westbrook area (34%) and approximately equal number classified to the other spawning stocks (Niantic River 24%, Thames River 21%). Peak entrainment of Niantic River larvae was in mid-April. Peak entrainment of Thames River larvae was in late May, early June, while peak entrainment of Westbrook larvae occurred in early May at the same time of peak entrainment into the Power Station (Table 7).

Table 7. Classification of entrained larvae to known nursery areas using the trained neural network. Classification (values are expressed as percentage of total)

Collection Date	Niantic River	Thames River	West-brook	Not Niantic River	Not Thames River	Not West-Brook	Un-known
3.28.01	46.2	7.7	15.4	23.1	0	7.7	0
4.09.01	14.6	29.2	34.8	6.7	7.9	3.4	3.4
4.16.01	29.5	22.7	25.0	6.1	6.1	9.1	1.5
4.23.01	35.6	23.3	21.7	5.0	2.2	11.7	0.6
4.30.01	27.2	15.4	33.3	8.0	9.9	4.3	1.9
5.07.01	16.2	14.0	51.5	9.6	5.9	1.5	1.5
5.14.01	17.2	13.8	46.0	9.2	5.7	4.6	3.4
5.21.01	21.7	30.4	31.9	10.1	0	4.3	1.4
5.28.01	18.8	29.2	29.2	4.2	10.4	2.1	6.3
6.04.01	6.9	24.1	48.3	17.2	3.4	0	0
6.11.01	18.5	18.5	37.0	18.5	7.4	0	0
6.17.01	23.1	38.5	7.7	7.7	7.7	15.4	0
% of all sampled larvae	24.1	21.0	33.5	8.1	5.8	5.7	1.8

Classification to one of the known spawning areas required at least a 0.75 confidence. Classification to the not-spawning area columns required that one of the spawning areas be classified as having <0.05 confidence of being the spawning area for that larvae. The unknown classification was for larvae that had equal confidence to belong to any of the spawning groups.

Juveniles that were collected in 2000 & 2001 from the Niantic River were compared to the tested spawning stocks (Table 8). Once again, very few juveniles could not be assigned to one of the 1st six groups. There was no significant difference in the classification of the juveniles collected in early or late summer of both years. LR station, located near the mouth of the river, had initially fewer juveniles classified to the Niantic spawning stock in June 2000 than the WA site that is more upriver. However less difference was seen in September 2000 or in 2001. The great majority of juveniles were classified to the Niantic River and Westbrook areas and not to the Thames River. Also of interest was the size of juveniles assigned to source populations, i.e., were larger juveniles produced within the Niantic River as opposed to smaller fish entering the River from other areas. A one-way analysis of variance indicated no significant difference in the juvenile size by year, month, and station except in September 2000 at the lower river site, where juveniles originating from Westbrook were significantly larger (mean of 43 mm) than those from the Niantic (36 mm) or Thames Rivers (35 mm).

Table 8. Classification of juvenile winter flounder to nursery areas using the trained neural networks.

	Classification (values are expressed as percentage of total)							
	June, 2000 LR	September 2000 LR	June, 2000 WA	September 2000 WA	June, 2001 LR	September 2001 LR	June, 2001 WA	September 2001 WA
Niantic River	21	23	51	26	20	28	16	17
Thames River	11	10	11	29	24	9	13	11
West Brook	40	37	17	37	27	36	44	33
Not Niantic	5	10	0	0	16	5	16	6
Not Thames	14	7	11	0	4	17	9	17
Not West Brook	7	13	6	6	9	5	2	11
Unknown	2	0	3	3	0	0	0	6

Conclusions & Discussion:

Previous work suggests that there are discrete breeding stocks of winter flounder, based on morphometric, meristic, tagging studies and other factors (Pearcy 1962a, 1962b, Berry et al., 1965). Work over the past few decades by the Connecticut Department of Environmental Protection has suggested that distinct spawning and nursery areas for winter flounder exist within LIS (Howell et al., 1999). One such nursery area is in the Niantic River that is nearby to the Millstone Power Station. In general, marine fish show less genetic differentiation than freshwater or anadromous fishes since marine environments are less fragmented than freshwater environments (Carvalho, 1994; Ward et al., 1994). Marine organisms with a planktonic phase have a high potential for physically and biologically mediated dispersal. Nonetheless, evidence does suggest that larval retention (Jordan et al., 2000), cohort fidelity (Sinclair 1988), geographical structures and impediments (Ruzzante et al., 1998) and natal homing instincts (Nielsen et al., 1999) may limit gene flow. Marine species such as cod, hake, herring and squid have previously shown little genetic population differentiation by allozyme markers and thought to be homogeneous over large geographical ranges. Recent examination with microsatellite loci has revealed fine levels of population structure (Bentzen et al., 1996; O'Connell et al., 1998, Lundy et al., 1999, Shaw et al., 1999).

Microsatellite loci form a class of highly polymorphic and informative regions of chromosomal DNA that have found great usage for studies of intra-specific population structures, as well as hybridization events, linkage mapping, paternity testing and pedigree analysis (Hughes & Queller et al., 1993, Roy et. al., 1994, Dowling et. al., 1997). Although statistically significant genetic differences do not always have biological significance (Waples 1998) they can play an

important role in fishery management issues or in instances of efforts to recover commercial fishing industries.

During normal Station operations, cooling water withdrawn by the Station causes the entrainment and death of millions of winter flounder larvae. The issue of plant impact due to winter flounder larval entrainment has been addressed by population dynamic modeling (Lorda et al., 2000). This modeling has focused on the Niantic River winter flounder stock and among the information required is an estimate of the annual reproductive output removed by entrainment. This fraction was determined using an indirect method (the mass-balance model). However, the present genetic analysis provides a more direct quantitative estimate of entrainment loss by source population. Thus, larvae in early developmental stages were collected from areas known to be near spawning grounds. The young stage 1 & 2 larvae appear to have limited geographical dispersal and hence likely retain genetic differences. Comparison of genetic differences between these groups demonstrates a relatively high degree of difference (Nei's genetic difference >0.05). This genetic differentiation is geographically based with the greatest difference seen between the Westbrook and the Thames River source areas that are separated by 20.5 miles. Though only separated by 5 miles, the Thames and Niantic River source areas had relatively substantial genetic separation between them (Nei's genetic difference = 0.036), suggesting that one or more factors might be limiting gene flow. The same genetic differences were seen in larvae collected in 2000, suggesting a temporal stability to these genetic differences.

There is no apparent geographical or physical barrier to gene flow between these areas so natal homing instincts or selective pressures may be responsible. These differences were sufficient to provide the resolution to assign entrained larvae or older, settled juveniles to the tested populations. Many different approaches have been used to assign individuals of unknown origin to populations based on the genetic distance between individuals and populations (e.g., neighbor-joining trees, Estoup et al., 1998, likelihood of the multi-locus genotype and Bayesian, Cornuet et al., 1999). A key component of any of these approaches is that they have the ability to correctly assign individuals, but a common drawback is that if the origin of the individual is not represented in the reference populations, most methods will still designate a wrong population of origin (Cornuet et al., 1999). Many approaches are based on two explicit assumptions that all loci are at Hardy-Weinberg equilibrium and at linkage equilibrium. Other constraints are the levels of differentiation between tested populations; the ability to sample all, or virtually all of the potentially contributing stocks, the temporal stability of the microsatellite markers and a large sample size that contains representation for all donor populations (Letcher and King, 1999; Smouse et al., 1990). Newer approaches have attempted to overcome these limitations (e.g. Bayesian) and maximizing the accuracy of assignment depends in part on the F_{ST} values, population sizes and loci number. The large differences in F_{ST} values among tested reference populations in this work, coupled with large population sizes (on average 150 individuals) and loci with large numbers of alleles (135) should allow for the maximal accuracy in assignment to reference populations. It is clear that we have not sampled from all of the possible donor stocks in LIS or surrounding areas, but focusing on a smaller geographical range across which all stocks can be sampled adequately, minimizes this problem.

Recently, several investigators have begun to use non-supervised or artificial neural networks (ANN or TNN) to assign individuals to populations based on genetic differences (Brosse et al., 1999; 2001; Wu-Catherine, 2000). These neural networks are trained on populations with known genetic differences and then applied to unknown individuals. Neural networks make no prior assumptions about the characteristics of the training sets and develop algorithms that maximize its

ability to correctly identify unknown individuals to populations. These ANN have been used in a wide range of areas including assessments of fish abundance and spatial occupancies (Brosse et. al., 1999).

The TNN used in this work was trained on microsatellite data generated from the populations found in the Niantic, Thames and Westbrook areas. To test the accuracy of this approach for correct assignment of individuals to populations, confidence values were generated and classification was compared to neural networks trained on randomized (and incorrect) training sets (i.e., the RNNs). The RNNs lost all ability to classify individuals and classification was essentially random. The TNNs classified individuals with high accuracy. A confidence value below 0.75 was the lowest confidence used to assign an individual to a source population. Even when the TNN could not assign an individual to a specific population with at least 0.75 confidence, it was capable of determining that an individual was not from a specific population (<0.05 confidence). When the TNN was applied to the entrained larvae and collected juveniles, the assignments, namely that not all of the entrained larvae were from the Niantic River stock, are in agreement with other approaches (e.g., mass-balance model based on larval dispersal). Previous mass-balance approaches (NUSCO, 2000) indicated that during 1984-98, about 12% to 59% of the entrained larvae were from the Niantic River with a long term average of 25.4% that is in agreement with the results seen here (24.1%). Previous work has also suggested that larval entrainment from the Niantic River area is highest earlier in spring. This likely occurs because this area is closest to the Station and larvae displaced from the River make up a greater proportion of all larvae found in Niantic Bay. As the season progresses more larvae from other sources are transported by tidal currents into this area and locally produced larvae are less dominant. Large assignments were made to the tested population that lies 15 miles to the west near the Connecticut River. The Thames River, which is 5 miles to the east, contributed far fewer larvae. This suggests that currents and tidal flow transport winter flounder larvae in April and May predominantly from a west-to-east direction. This is supported by the fact that the contribution of the Thames River population is also highest June.

The assignment of larvae to the Westbrook (i.e., Connecticut River) area reaches a peak in mid-May, when the contribution of larvae from the Niantic River has reached a nadir. The peak entrainment in the Station occurs in late April & early May when the contribution from the Westbrook area is greatest.

Over 80% of the larvae could be definitively assigned to one of the tested populations with at least a 0.75 confidence value. Of the remaining 20%, the TNN could determine that they did not belong to one of the populations and the confidence values were roughly divided between the remaining two. There were very few larvae (2%) that could not be assigned to the tested populations or were known not to have come from a specific population.

This approach is also able to classify the most likely source population of young-of-the-year juvenile flounder. Though only the genetic differences between larvae collected in 2001 were used in the TNN, we had previously characterized larvae in 2000 and found the genetic differences between the source populations to be almost identical as they were in 2001. Juvenile flounder of the 2000 & 2001 year class that were collected in July and September of each year from two sites in the Niantic River were then assigned to the tested populations with the TNN. The lower river site had initially a smaller proportion of juveniles assigned to the Niantic River than the mid-river site, where there were more juveniles resulting from the Thames River. Also, there were substantial numbers of juvenile flounder from the Westbrook are that were captured in the Niantic River. Overall, in 2001, 21% of the juveniles were classified as of Niantic River origin, 15% from the Thames River, 25% from the Westbrook area and the source of 28% of the juveniles could not be

determined. These results clearly demonstrate that though the stage 1 larvae found in up-river spawning areas have substantial genetic differences with larvae from other spawning areas, the young-of-the-year that settle in the Niantic River are from a wide geographic area.

In terms of fisheries management, this approach allows for the estimation of the impact of a commercial operation (i.e., the Power Station) on specific flounder spawning groups and also allows for the direct linkage between a flounder spawning stock and recruitment to juveniles. Both of these factors are critical components of any fishery management plan that attempts to maintain a commercially viable winter flounder population in LIS.

Future experiments:

- If these experiments were continued for another year, I would recommend that we do so at the same level of effort as this year. Collection for an additional year will allow us to determine the variance in the genetic difference between the spawning populations as well as the variance in the entrainment of larvae. If this information is correlated with hydrodynamic information (tides, currents, spring thaws, etc.) and applied to current plant operations, then it might be possible to predict the entrainment from spawning areas in future years with a high level of confidence.
- With information from a third year, it will be possible to determine Ne, the effective adult spawning population in the different river areas. Ne refers to the amount of adults that contribute the genes to 95% of the population and is a useful value for fishery management issues and identifies the number of females producing the larvae found in each river system.

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Northeast Utilities
Environmental Laboratory

April 24, 1992

Waterford, Connecticut
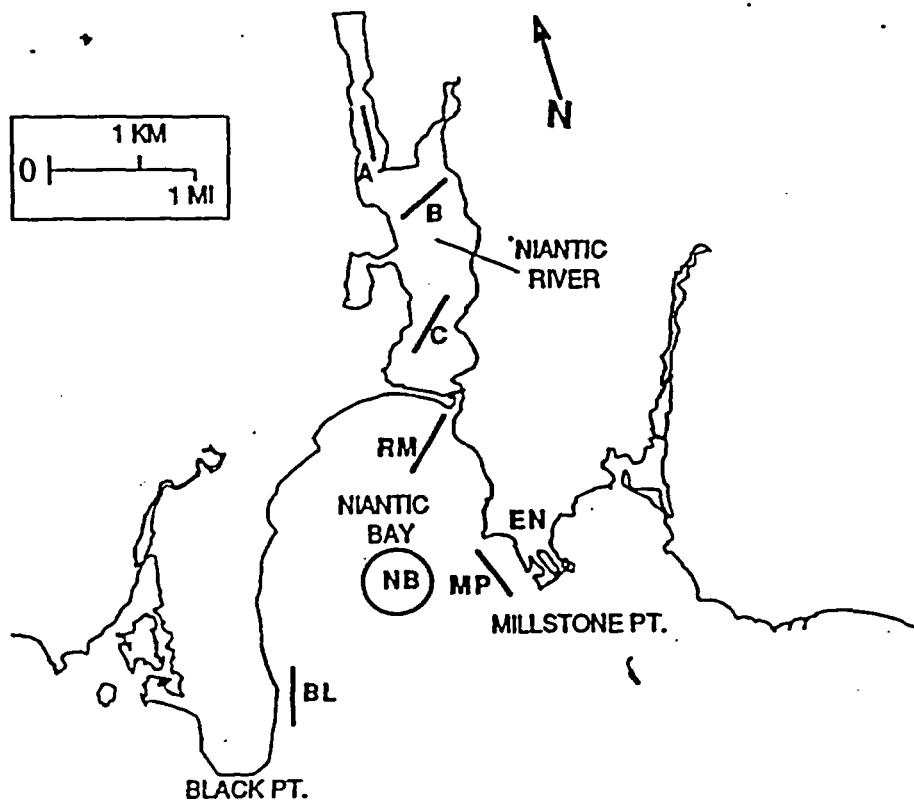
Fig. 3. Location of stations sampled for larval winter flounder during 1991.

using a single GO flowmeter mounted in the center of each bongo opening. The sampler was towed at approximately 2 knots using a stepwise oblique tow pattern, with equal sampling time at surface, mid-depth, and near bottom. The length of tow line necessary to sample the mid-water and bottom strata was determined by water depth and tow-line angle measured with an inclinometer. The nets were towed for 6 minutes at stations A, B, and C (filtering about 120 m^3) and 15 minutes at station NB (filtering about 300 m^3). One of the duplicate samples from the bongo sampler was retained for laboratory processing.

The larval winter flounder sampling schedule for Niantic River and Bay was based on knowledge gained during previous years and was designed to increase data collection efficiency while minimizing sampling biases (NUSCO 1987). Larval sampling at the three Niantic River stations usually started in mid-February. From then through the end of March, daytime tows were conducted within 1 hour of low slack tide. During the remainder of the season, until the disap-

pearance of larvae at each station, tows were made at night during the second half of a flood tide. From 1983 through 1990, sampling was conducted 2 days a week. In 1991, sampling was reduced to 1 day a week (NUSCO 1991a). At NB, single day and night tows were made every two weeks during February and at least once a week from March through the end of the larval winter flounder season. During 1991, all day collections in March were made during a flood tide and in April and May all night collections were taken during a flood tide. Jellyfish medusae at the three river stations were removed (1-cm mesh sieve) from the samples and measured volumetrically to the near-est 100 ml.

Three additional stations in Niantic Bay were sampled during 1991 to determine larval winter flounder abundance and spatial distribution relative to tidal currents. Station RM was located south of the mouth of the Niantic River, MP west of Millstone Point, and BL east of Black Point (Fig. 3). Six-minute stepwise oblique tows were made with the bongo

Materials & Methods

sampler described above. Sampling was conducted within one hour from the time of maximum ebb and flood tidal currents twice a week from March through May. Collections were made during daylight in March with 202-µm mesh nets and during night-time in April and May with 333-µm mesh nets. For station MP, neither ebb nor flood samples collected on March 8 were processed due to heavy detrital loads.

During 1991, the vertical distribution of yolk-sac (Stage 1) winter flounder larvae in the Niantic River was determined using a pump sampler (Fig. 4). The sampler was designed to collect larvae from discrete depths and remove them before they passed through the pump, which would have destroyed yolk-sac winter flounder larvae. The intake of the sampler consisted of four 10.3-cm diameter inlets connected to a 15.4-cm hose; the inlet openings were perpendicular to the bottom. The intake could be raised and lowered to sample discrete depths. The 15.4-cm hose was connected to the top of the net chamber located aboard the research vessel. The net chamber was fabricated from fiberglass and had a diameter of 0.6 m, was 1 m deep, and was designed to withstand a perfect vacuum.

A 202-µm mesh net was attached to a net ring within the net chamber. Seawater was filtered though the net, which removed larvae, and exited through the bottom of the net chamber via a 7.7-cm hose connected to a gasoline-powered pump (Pacer Pumps, Model SE3SLL). Water volume was measured with an electronic in-line flowmeter (Omega Engineering, Inc.) located in the pump discharge pipe; flow rate and total sample volume were registered on a remote readout box. The pump capacity was about 1 m^3 per minute and total sample volume ranged from about 5 to 10 m^3. During sampling, the research vessel was anchored from both the bow and stern to prevent movement; this stability was particularly important while sampling at the sediment-water interface. A total of 12 sets of samples were collected during February and March at the three Niantic River stations (Fig. 3). Each set consisted of collections at the surface, mid-depth, near bottom (approximately 0.3 m above the bottom), and at the sediment-water interface. Five sets of samples were collected at station A, six at B, and only 1 at C. Station C was located in the navigational channel, where strong tidal currents made sampling difficult.
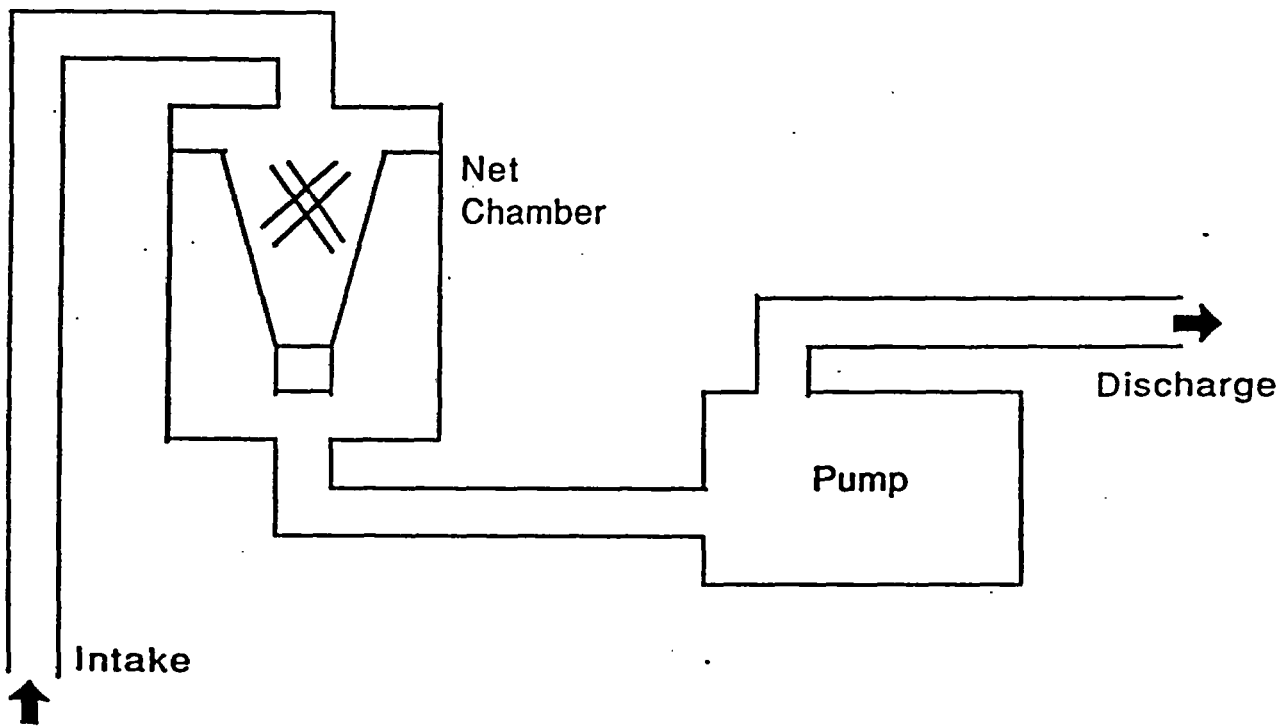

Fig. 4. Schematic of the pump sampler used to collect winter flounder larvae in the Niantic River during 1991.

years in both the river and bay, except for the river in 1988. Dates of peak abundance for older larvae were similar in both the river and bay and because those for Stage 2 larvae differed considerably, this suggested that most larvae were probably flushed from the river during Stage 2 of development.

Previously, it was shown that water temperature may affect the rate of development for winter flounder larvae, where growth and development were positively related to temperature (NUSCO 1991b). The warmer than normal water temperature during the larval season in 1991 (Table 6) and the early peaks of Stage 3 and 4 larvae in the river and bay during 1991 suggested a relationship between water temperature and the rate of larval development. The mean March-April water temperature in the bay (determined from a continuous recorder in the intakes of Units 1 and 2) in 1991 was 6.8°C (95% CI of 6.5-7.2°C), the highest of the preceding 15 years (1976-90), which had a mean of 5.1°C (95% CI of 5.0-5.2°C). Buckley et al. (1990) reported that egg incubation time was inversely related to water temperature during oocyte maturation and egg incubation. The early peaks of Stage 1 and 2 larvae in the river during 1990 was related to higher than average February water temperatures (NUSCO 1991b). The average February 1991 water temperature (4.8°C: 95% CI of 4.6-4.9°C) was even higher than found for 1990 (4.3°C: 95% CI of 4.1-4.5°C), but the dates of peak abundance for Stage 1 and 2 were near the average for the 9-year period. Based on the results from field data, it appeared that water temperature affects larval developmental rates, but its effects on oocyte maturation and egg incubation rates was not clear.

During 1991, a special sampling program was conducted in Niantic Bay to examine larval winter flounder temporal and spatial distribution and abundance relative to tidal stage. Three new stations (RM, MP, and BL) were sampled from March through May with paired ebb and flood tidal stage collections taken during times of maximum tidal current; collections at station NB were made only during a flood tide. A comparison of abundances based on the α parameter from the Gompertz function (Eq. 2) indicated large differences between ebb and flood collections at the three new stations (Table 15). Two distinct groups were evident on the basis of abundance. Larvae were most abundant for RM-flood, MP-flood, and BL-ebb collections, with α values ranging from 4,225 to 5,322. Similarly, lowest abundances were found for RM-ebb, MP-ebb, and BL-flood, with α values ranging from 1,865 to 2,124. The NB-flood collections were also within this lower abundance range. The 95% confidence interval indicated good precision of the abundance estimates, except for both ebb and flood collections at station RM. The low of precision in estimating the other two parameters (p and κ) was also evident for RM. Many of the samples collected at RM had heavy detrital loads, particularly during ebb collections, which may have affected the quality of sample processing.

Abundance curves were constructed based on the Gompertz density function (Eq. 3) to examine temporal abundance at each bay station (Fig. 14). Due to the low precision of parameter estimates for station RM, only limited interpretations were made concerning temporal changes at this location. The estimated abundance for RM-flood collections began to exceed RM-ebb in early March and continued throughout the season. MP-flood abundance was greater than MP-ebb starting on March 23. In contrast, BL-ebb abundance exceeded BL-flood beginning on April 5. The shape of the abundance curves and density estimates were similar for MP-ebb, BL-flood, and NB-flood (Fig. 15).

Since tidal current patterns in Niantic Bay could affect larval abundance, the examination of these patterns may provide some insight into the differences

TABLE 15. Larval winter flounder abundances and 95% confidence intervals for ebb and flood tide collections at stations NB, RM, MP, and BL as estimated by the α parameter from the Gompertz function.

Station	Ebb		Flood	
NB	-[a]		1,889	(1,829-1,950)
RM	2,124	(948-3,299)	5,141	(2,374-7,908)
MP	1,865	(1,688-2,044)	4,225	(3,464-4,986)
BL	5,322	(4,719-5,924)	1,962	(1,688-2,236)

[a] Ebb tide was not sampled.

Results
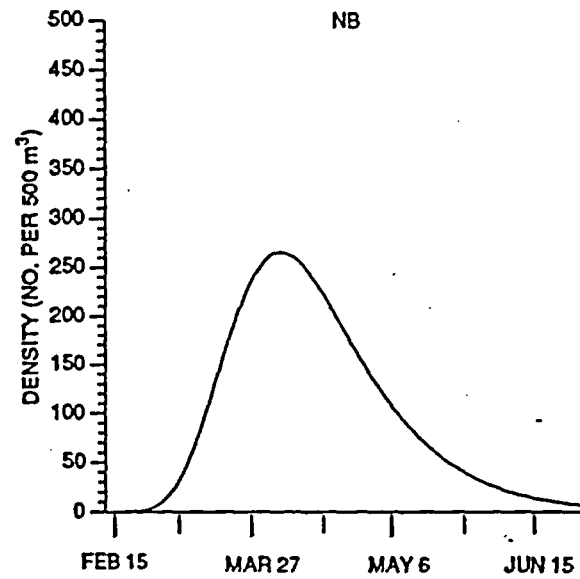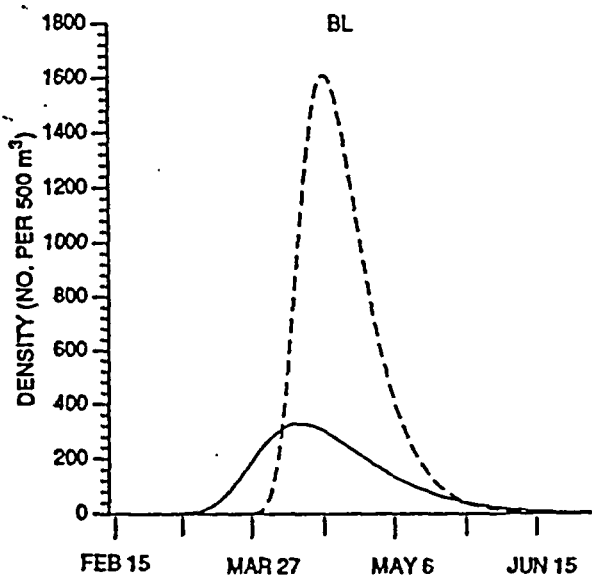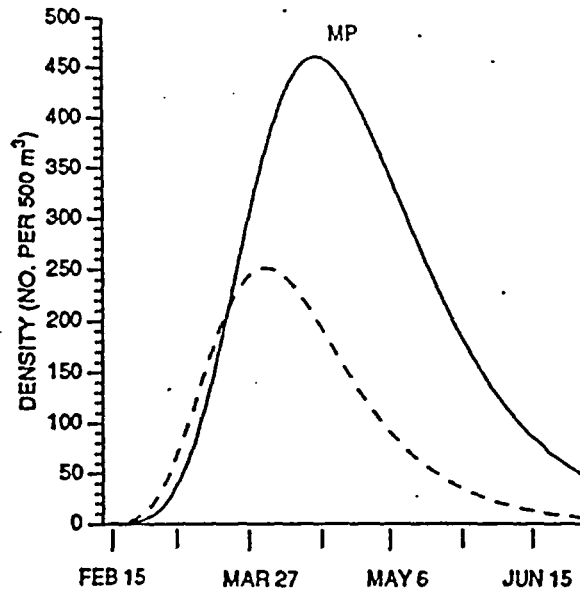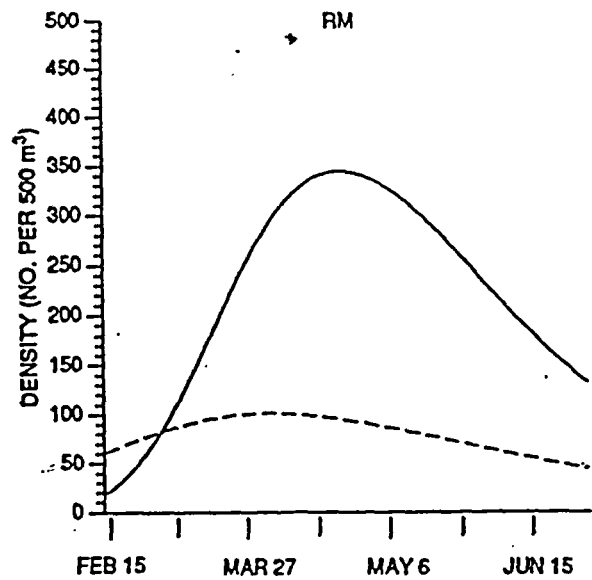
Fig. 14. Abundance curves estimated from the Gompertz density function for larval winter flounder during an ebb and flood tidal stages at stations RM, MP, BL, and NB in 1991.

in density estimates between ebb and flood collections at the various stations. Hydrodynamic modeling has provided simulations of tidal currents in Niantic Bay during maximum ebb and flood currents (NUSCO 1976: Figs. 4.2-2 and 4.2-4). In addition, several current drogue studies were conducted during 1991 to verify the model simulations (see the Niantic Bay Current Studies section in this report). Based on the hydrodynamic model simulations and verification with current drogues, generalized current flow patterns were
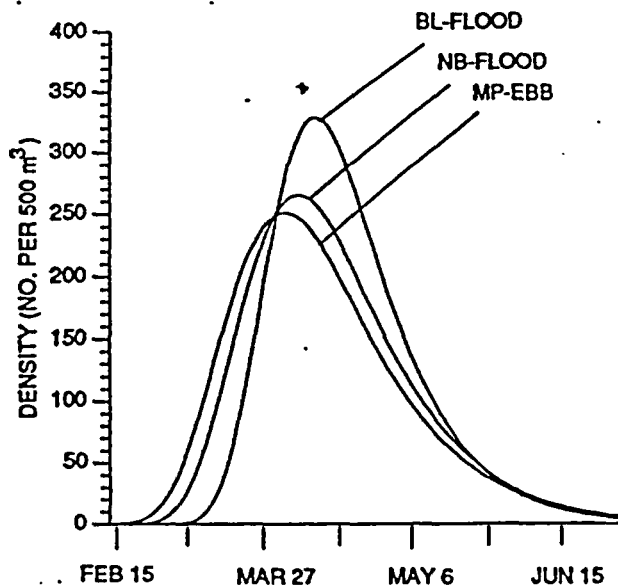
Fig. 15. Comparison of abundance curves estimated from the Gompertz density function for larval winter flounder during selected tidal stages at stations MP, BL, and NB.

prepared for maximum ebb and flood currents (Fig. 16). During maximum flood current some of the water mass flowing westward south of Millstone Point enters Niantic Bay, flowing north by the MNPS intakes and towards the Niantic River mouth. The remaining water mass continues westward until being deflected towards the southwest by Black Point. During maximum ebb current, some of the water mass flowing eastward south of Black Point enters Niantic Bay and flows to the northeast. The flow then turns to the southeast, passes MNPS intakes, and exits the Niantic Bay to the east.

The circulation patterns and differences in larval abundance between ebb and flood tides at stations MP and BL indicated that as the larval season progressed into April, large numbers of larvae entered Niantic Bay from LIS. Collections with the greatest larval abundance were MP-flood and BL-ebb. In both collections, the primary source of water entering the bay was from LIS. In contrast, the lowest larval densities occurred during MP-ebb and BL-flood collections. For these collections the water sampled had entered from LIS, but had flowed across the bay and most likely mixed with bay water, possibly diluting the greater densities of larvae entering from LIS. The abundance during NB-flood collections were similar to BL-flood and MP-ebb (Fig. 15), suggesting a similar dilution of LIS water in the bay. Previous 24-hour tidal stud-

ies at station NB showed no tidal-related differences in winter flounder larval abundance (NUSCO 1989a).

Developmental stage-specific abundances were compared at stations RM, MP, and BL on the basis of cumulative weekly geometric means, an approximation for the α parameter in the Gompertz function (Fig. 17). Stage 1 larvae were most abundant at station RM, with the Niantic River the most probable source of this early developmental stage. The abundance of Stage 2 was more homogenous in the bay, except for RM-ebb collections. Stage 3 abundance was much greater than Stage 2 and collections by tidal stage indicated that more were entering the bay from LIS (MP-flood and BL-ebb) than were being flushed out of the bay to LIS (MP-ebb and BL-flood). A similar pattern at stations MP and BL was evident for Stage 4 larvae.

The results of this special bay-wide sampling in 1991, taking into account tidal circulation patterns, provided some insight into the sources of winter flounder larvae in Niantic Bay. Early in the larval season the primary source appeared to be the Niantic River, but as the season progressed the major source was LIS. Because this change in sources occurred later in the season, these older larvae could have originated from spawning stocks both east and west of the Niantic Bay and transported by tidally currents to the Millstone area.

Development and growth

The length-frequency distribution for each stage has remained fairly consistent since developmental stage determination began in 1983 (NUSCO 1987, 1988c, 1989a, 1990a, 1991b). Stage-specific length-frequency distributions by 0.5-mm size-classes in 1991 showed a separation in predominant size-classes for the first four developmental stages (Fig. 18). Stage 1 larvae were primarily in the 2.5 to 3.5-mm size-classes (96%), Stage 2 were 3.0 to 4.0 mm (84%), Stage 3 were 4.5 to 7.5 mm (82%), and Stage 4 were 7.0 to 9.0 mm (87%). These consistent results from year to year indicated that developmental stage and length of larval winter flounder were closely related. This agreed with laboratory studies on larval winter flounder which showed that there were positive correlations between growth and developmental rates (Chambers and Leggett 1987; Chambers et al. 1988). This relationship allowed for the estimation of developmental stage from length-frequency data.
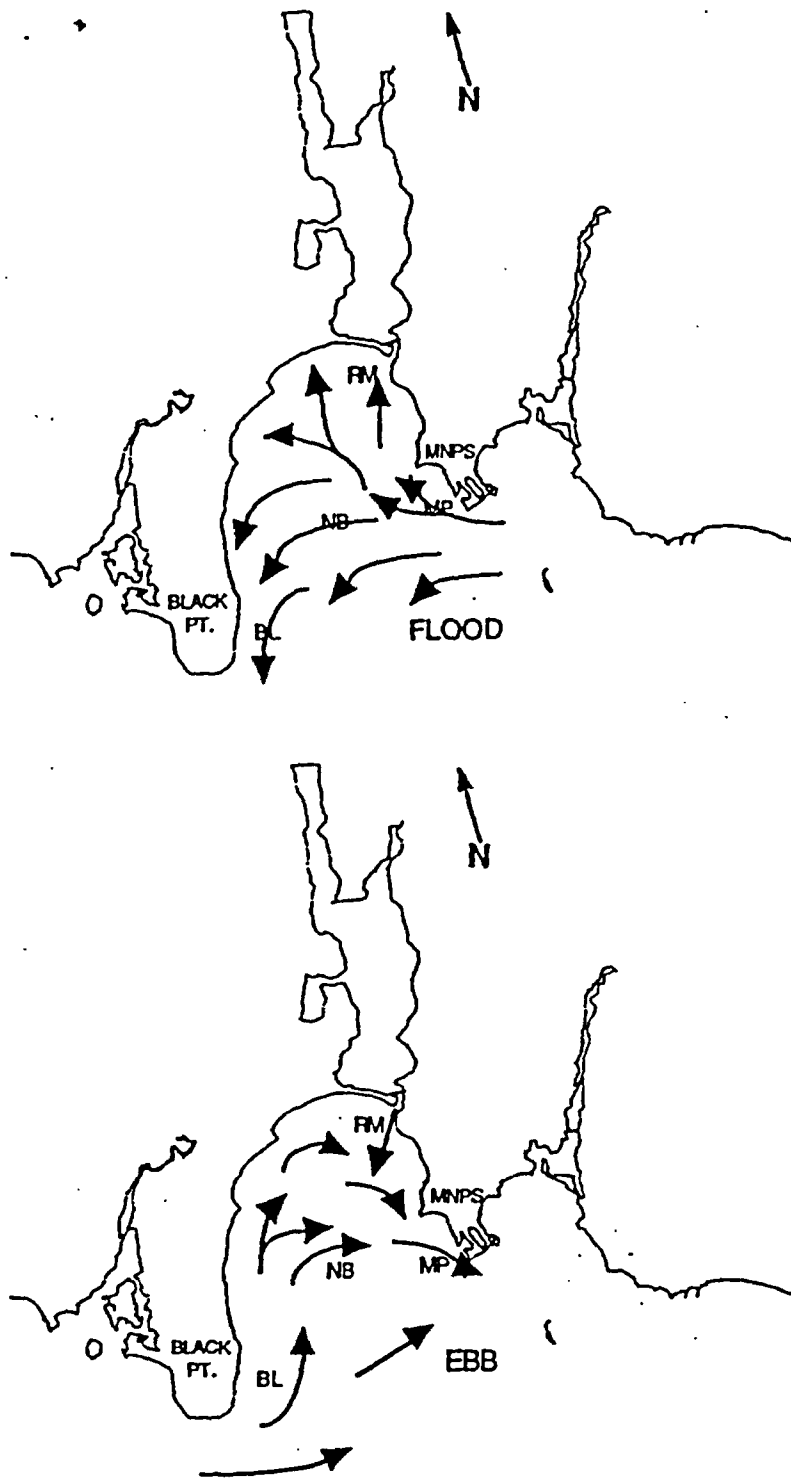
Fig. 16. Generalized current patterns in Niantic Bay during maximum flood and ebb current, adapted from hydrodynamic model simulations (NUSCO 1976: Figs. 4.2-2 and 4.2-4). Also shown are larval winter flounder sampling locations in Niantic Bay during 1991.
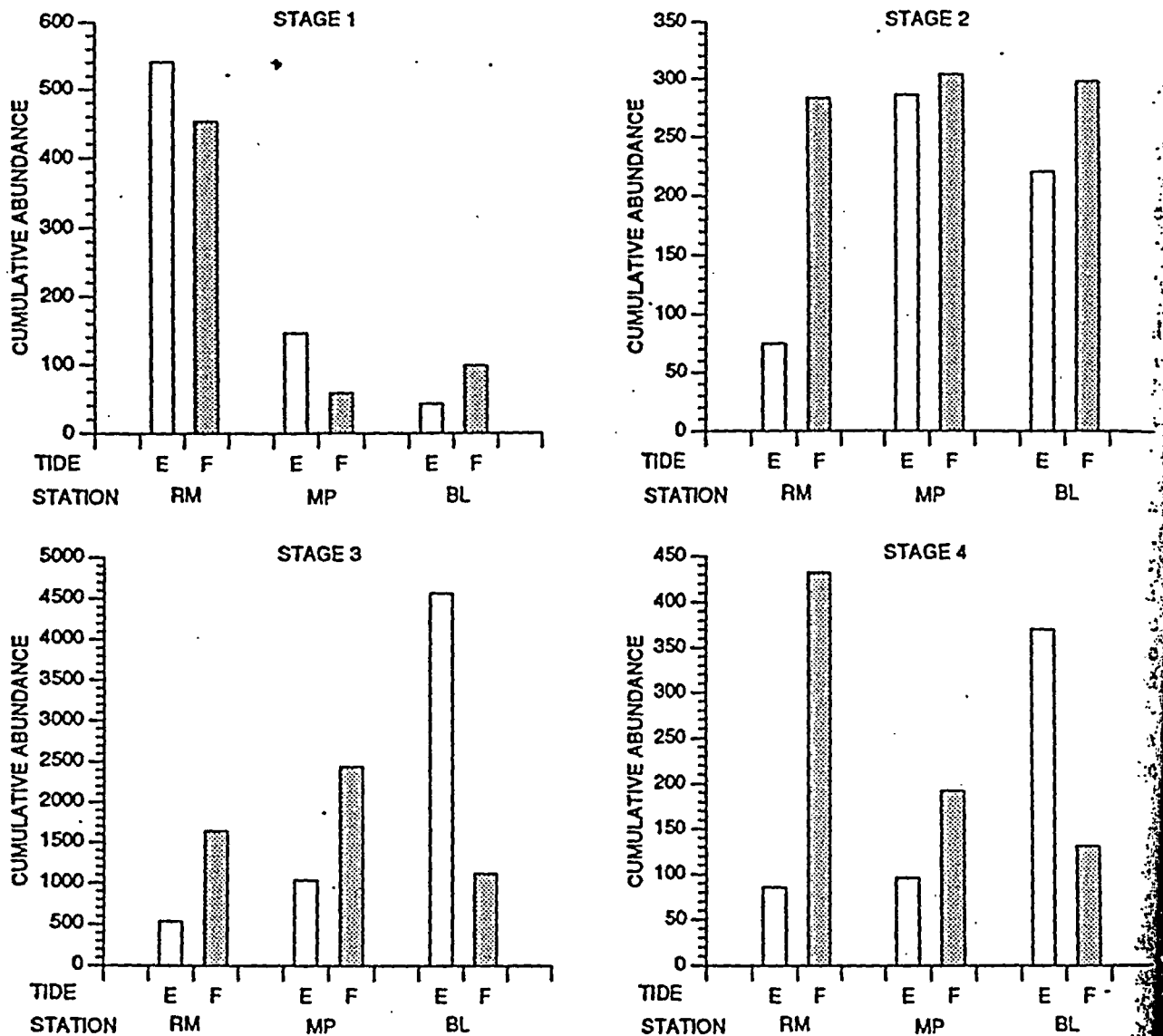
Fig. 17. Cumulative density by developmental stage for larval winter flounder collected during ebb and flood tidal stages at stations RM, MP, and BL in 1991.

The length-frequency distributions of larvae (all stages combined) collected in the Niantic River (stations A, B, and C combined) were quite different than for Niantic Bay (stations EN and NB combined) in 1991 (Fig. 19). The differences in size-class distribution between the two areas were similar to previous findings reported in NUSCO (1987, 1988c, 1989a, 1990a, 1991b) and consistent with the spatial distribution of developmental stages (Figs. 11 and 12). Smaller size-classes predominated in the river during 1991, which had about 70% of the larvae in the 3.5-mm and smaller size-classes. In contrast, more than

85% of the larvae in the bay during 1991 were in the 4.0-mm and larger size-classes. The slight increase in frequency for the larger size-classes in the river has been apparent in some previous years (NUSCO 1987, 1988c, 1989a, 1991b) and was additional evidence that some older larvae were imported to the river.

Length-frequency data from entrainment collections (station EN) were used to estimate larval winter flounder growth rates for Niantic Bay; these data were examined because a 16-year time-series was available. Weekly mean lengths during a season formed a sigmoid-shaped curve (NUSCO 1988c). The line

Monitoring the Marine Environment of Long Island Sound at Millstone Nuclear Power Station 1988 Annual Report

Northeast Utilities
Environmental Laboratory

April 19, 1989

Waterford, Connecticut
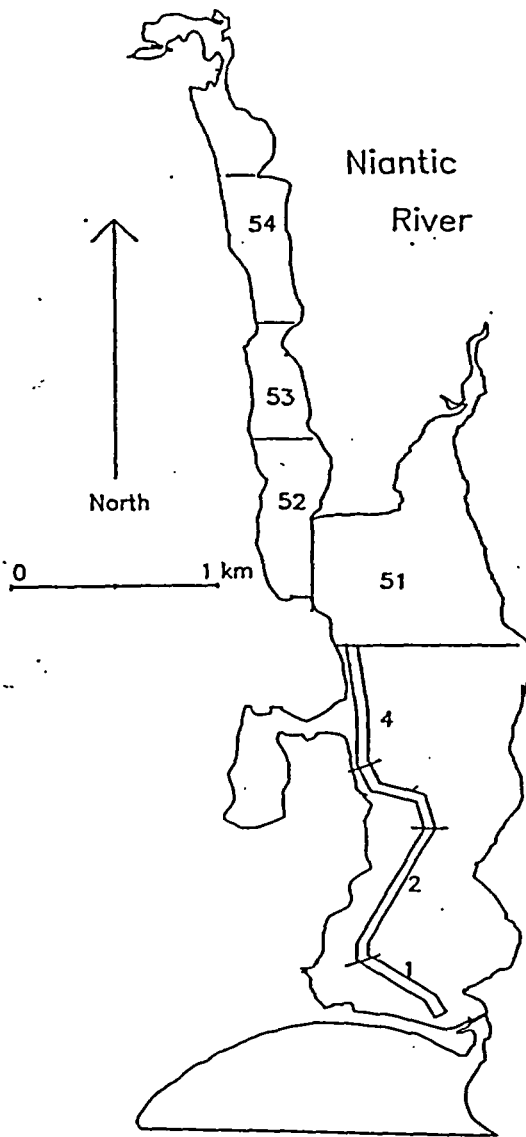
Fig. 2. Location of stations sampled for winter flounder during the spawning season in the Niantic River during 1988.

have been classified into various length and sex groupings, depending upon the year; at minimum, all fish caught can be classified as smaller or larger than 15 cm. Since 1977, the sex and reproductive condition of the larger winter flounder have been determined either by observing eggs or milt or by the presence (males) or absence (females) of ctenii on the caudal peduncle scales of the left side (Smigielski 1975). Fish larger than 15 cm (1977-82) or 20 cm (1983-88) were marked with a number or letter made by a brass brand cooled in liquid nitrogen and were then released. The mark was changed weekly and fish recaptured were noted and remarked with the brand designating the current week of sampling.

Larval winter flounder

Winter flounder larvae have been routinely sampled in Niantic River at stations A, B, and C since 1983, and in Niantic Bay at NB since 1979 and at EN (entrainment sampling) since 1976 (Fig. 3). In addition, special studies were conducted during 1988 at the mouth of the Niantic River to examine larval export-import; 24-h sampling was conducted in mid-Niantic Bay and Twotree Island Channel to examine possible tidal and diel larval behavior; and a comparison of entrainment sampling densities was made at the discharges of Units 1, 2, and 3. The export-import studies and the 24-h sampling studies are described below. The comparison of entrainment sampling densities among the units was presented in the Fish Ecology Section of this report.

Collections in the river and at NB were made with a 60-cm bongo sampler with 3.3-m long nets towed at approximately 2 knots and weighted with a 28.2-kg oceanographic depressor. Volume of water filtered was determined using a single General Oceanics (GO) flowmeter (model 2030) mounted in the center of each bongo opening. A stepwise oblique tow pattern was used with equal sampling time at surface, mid-depth, and near bottom. The length of tow line necessary to sample the mid-water and bottom strata was based on water depth and tow-line angle measured with an inclinometer. Winter flounder larvae

containers aboard the survey vessel before processing. At least 200 randomly selected fish were measured to the nearest 0.1 cm in total length during each week of the survey in all years. Since 1983, all winter flounder larger than 20 cm have been measured and sexed. Fish not measured

Materials &
Methods
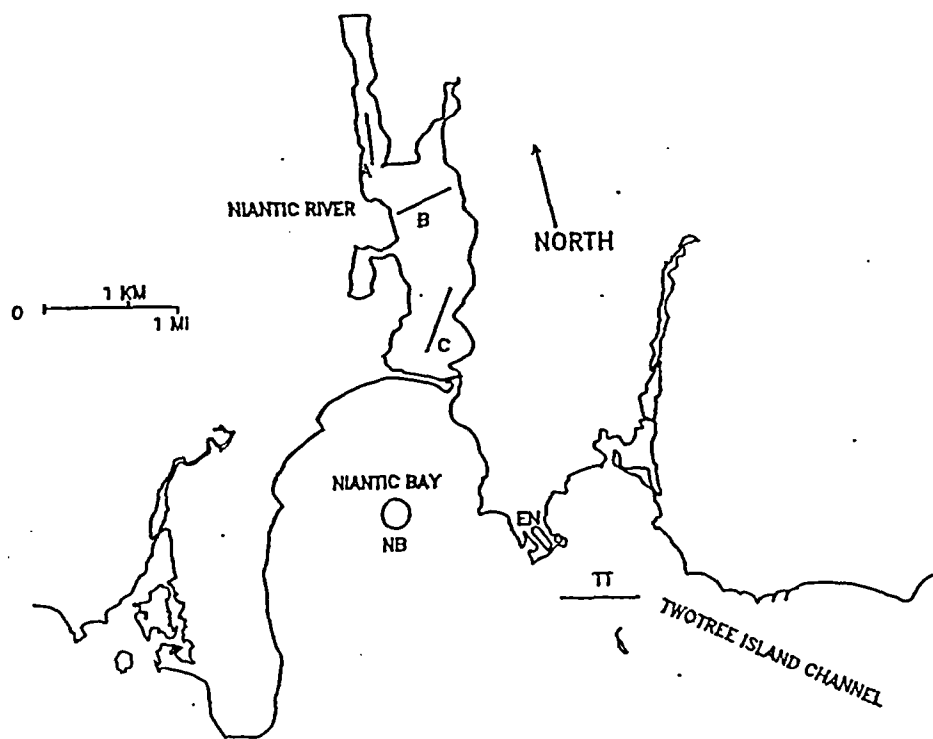
Fig 1   Location of stations sampled for larval winter flounder in 1988.

entrained by MNPS were collected at Units 1 and 2 discharge (station EN) using a gantry system to deploy a 1.0 x 3.6-m plankton net. Four GO flowmeters were positioned in the mouth of the net to account for horizontal and vertical flow variation; sample volume was determined by averaging the four volume estimates from the flowmeters.

All sampling at EN was conducted with 333-µm mesh nets. On the bongo sampler, 202-µm mesh nets were used from February 23 through the last week of March and 333-µm mesh nets during the remainder of the season. The bongo sampler was towed for 6 min at stations A, B, and C (filtering about 120 m³) and for 15 min at station NB (filtering about 300 m³). Generally, the net was deployed at EN for 5 to 6 min (filtering about 400 m³), but this varied depending

upon plant operations (number of circulating pumps). All ichthyoplankton samples were preserved with 10% formalin. At the three river stations, jellyfish medusae were sieved (1-cm mesh) from the sample and measured volumetrically (ml).

During the larval winter flounder season, sampling time and frequency varied by station and month. At EN, two replicate samples were taken during both daylight and at night once per week in February and June, and during four days and nights per week from March through May. Single bongo tows were made during the day and night at NB biweekly in February and at least once a week in March through the end of the larval winter flounder season. Sampling in the Niantic River did not start in 1988 until February 23 because of ice. From the start of sampling through

the end of March, single daytime tows at each station were made twice weekly within an hour of low slack tide. During the first three weeks of April, single day and night bongo tows were made twice weekly. Day samples were collected within an hour of low slack tide and night samples during the second half of a flood tide. During the remainder of the season, until the disappearance of larvae at each station, tows were made twice a week only at night during the second half of a flood tide. This sampling scheme, based on information from previous years, was designed to increase efficiency in data gathering and reduce sampling biases (NUSCO 1987a).

Larval export-import studies were conducted at the mouth of the Niantic River in 1988 on March 24, April 18, May 2, May 10, and May 17. Stationary tows were taken by mooring the boat to the Niantic River Highway Bridge in the middle of the channel. During a complete tidal cycle, samples were taken hourly except from 1 hour before to 1 hour after slack tidal currents. A bongo sampler with 202-μm mesh nets was used on March 24; 333-μm mesh nets were used on the other four dates. The bongo sampler was deployed off the side of the boat and was lowered and raised between the surface and near bottom continuously during the sampling period. Sampling duration varied from 6 to 15 min (depending velocity of tidal currents) to sample approximately 100 m^3 of water.

Two 24-h studies were conducted to examine diel and tidal effects on sample density at stations NB and TT on April 25-26 and May 4-5 (Fig. 3). At both stations, samples were collected approximately every 2 hours intervals through the two 24-h periods. Tow durations were 15 min using a bongo sampler with 505-μm mesh nets. Stepwise oblique tows were made to sample equally at the surface, mid-depths, and near bottom.

Post-larval age-0 and age-1 winter flounder

Annual surveys of post-larval young-of-the-year winter flounder in the Niantic River began in 1983 (NUSCO 1987a). Station LR has been sampled every year and WA since late 1984 (Fig. 4). Two Niantic Bay stations (RM and BP) were established in 1988. Each station was sampled weekly from late May through late September during daylight from about 2 hours before to 1 hour after high tide. Sampling ceased at the Niantic Bay stations in September when few or no young were present.

A 1-m beam trawl with interchangeable nets of 0.8-, 1.6-, 3.2-, and 6.4-mm bar mesh was used to catch age-0 winter flounder. Two tickler chains were added in late June of 1983 to increase catch efficiency, because older and larger young apparently were able to avoid the net without them (NUSCO 1987a). In 1983, triplicate tows were made using nets of increasing larger mesh as fish grew during the season. Since 1984, two nets of successively larger mesh have been used during each sampling trip to collect the entire available size range of young. A change to the next larger mesh in the four-net sequence was made when fish had grown enough to become susceptible to it; the larger meshes reduced the amount of detritus and algae retained. Two replicates with each of the two nets were made at all stations, deploying them in a random order. Distance was estimated by letting out a measured line attached to a lead weight as the net was towed at about 25 m per min. Tow length was increased from 50 to 75 and to 100 m as the number of fish decreased throughout the summer.

An abundance index of juvenile winter flounder during fall and winter was calculated by using catches from the trawl monitoring program; field sampling methodology is detailed in the Fish Ecology section of this report. Because data on juvenile fish abundance were available from about May of their birth year into April of the following year, juvenile indices were referred to as age-0 or

TABLE 9. Estimated dates of peak abundance of larval winter flounder for each developmental stage in the Niantic River and Bay.

Year	Stage 1	Stage 2	Stage 3	Stage 4
		Niantic River		
1983	Mar 5	Mar 15	Apr 18	May 1
1984	Mar 7	Mar 9	Apr 26	May 19
1985	Mar 12	Mar 16	Apr 28	May 15
1986	Feb 26	Mar 7	Apr 23	May 12
1987	Mar 10	Mar 15	Apr 22	May 9
1988	Feb 29	Mar 9	Apr 6	May 1
		Niantic Bay		
1983	-	Apr 7	Apr 24	May 10
1984	-	Apr 8	May 4	May 25
1985	-	Apr 1	Apr 28	May 18
1986	-	Apr 6	Apr 29	May 12
1987	-	Apr 5	Apr 25	May 16
1988	-	Mar 24	Apr 23	May 10

Predation may affect larval abundance and there are numerous accounts of jellyfish being predators of fish larvae. Several species of hydromedusae and the scyphomedusa, *Aurelia aurita*, prey upon herring larvae (Arai and Hay 1982; Moller 1984), and laboratory studies with cod, plaice, and herring have shown that the capture success by *A. aurita* increased with medusal size (Bailey and Batty 1984). Evidence of a causal predator-prey relationship on larvae of two European flatfishes (*Pleuronectes platessa* and *Platichthys flesus*) by *A. aurita* and the ctenophore, *Pleurobrachia pileus*, was reported by van der Veer (1985). Pearcy (1962) stated that *Sarsia tubulosa* medusae were important predators of larval winter flounder in the Mystic River, CT, and had greatest impact on younger, less mobile individuals. Crawford and Carey (1985) reported large numbers of the moon jelly (*A. aurata*) in Point Judith Pond, RI and felt that they were a significant predator of larval winter flounder. Medusae of the the lion's mane jellyfish (*Cyanea* sp.), prevalent in collections at station A, were suspected of being an important predator of larval winter flounder in the upper portion of the Niantic River (NUSCO 1987a). Marshall and Hicks

(1962) also reported that jellyfish were more abundant in the upper river. In addition, laboratory studies have shown that winter flounder larvae which contacted the tentacles of the lion's mane jellyfish were stunned and ultimately died, even if not consumed by the medusa (NUSCO 1988a). Similar to 1985 and 1987, jellyfish abundance at station A in 1988 was relatively low when compared to 1983, 1984, and 1986 (Fig. 15). Coincident with the low jellyfish abundances in 1985 and 1988 were the highest abundances of Stage 2 larvae at station A (Fig. 13). Although few Stage 2 larvae were taken at station A in 1987, their abundance was low at all stations, thus obsuring any observation on the effects of predation. No causal predator-prey relationship in the Niantic River has been established, but there is strong circumstantial evidence that the lion's mane jellyfish may be an important source of mortality for winter flounder larvae.

Sampling was conducted over 24-h periods on April 25 and May 4, 1988 at stations NB and TT to examine the effects of tidal stage and day-night collection on the sample density of winter flounder larvae. These sampling dates were selected

Results
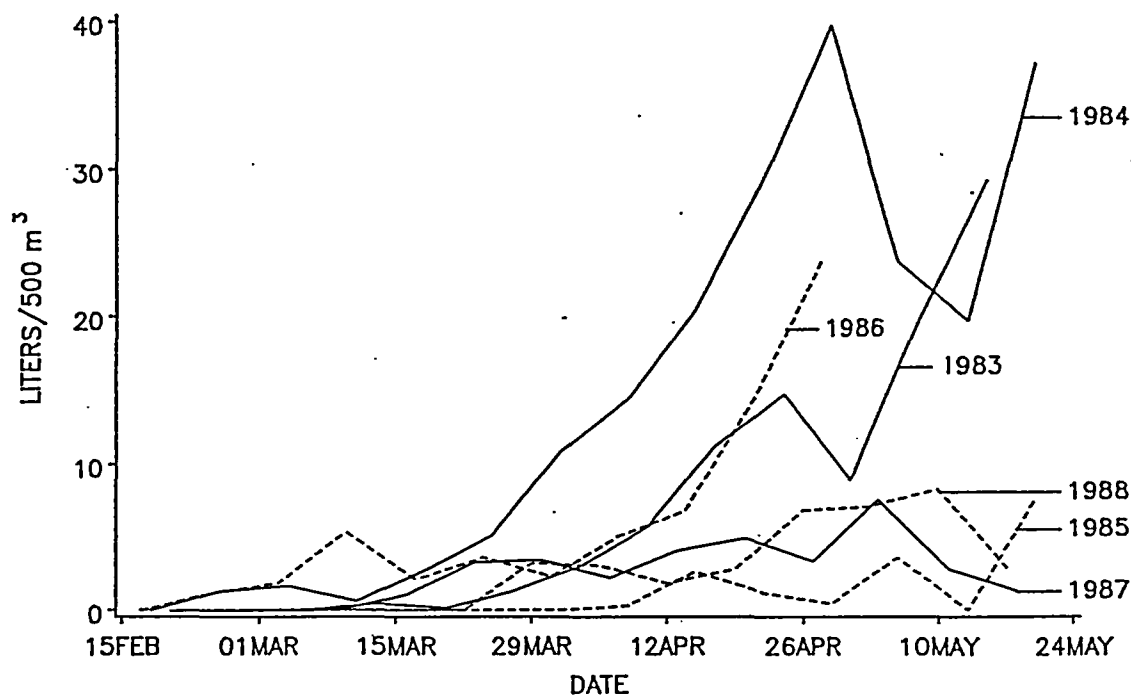
Fig. 15. Weekly mean volume (L per 500 m³) of *Cyanea* sp. medusae collected at station A in the Niantic River from 1983 through 1988.

to provide opposing tidal stages during the same time of day to discriminate between possible diel and tidal responses of larvae. Stage 3 larvae dominated the collections (95%) at both stations for the two dates. There was a large variation in sample densities during both sampling dates (Fig. 16). In general, densities were greater at night than during daylight and there was no apparent tidal influence on collection densities. Larval densities were significantly (p < 0.025) greater at NB for both dates combined and for each date seperately, tested with the Wilcoxon's signed-rank test (Sokal and Rohlf 1969) by pairing the samples collected within the same 2-h intervals at NB and TT. The seawater flow through Twotree Island Channel is large, and sample densities from station TT are probably more representative of larval winter flounder abundance found in LIS than collections taken in Niantic Bay because of the proximity of station NB to the Niantic River. Although den-

sities at station TT were lower than at NB, larval densities in Twotree Island Channel generally exceeded 100 per 500 m³, suggesting that there were large numbers of winter flounder larvae throughout LIS at this time of the season. Most likely, some of the large numbers of larvae found in LIS in the proximity of Millstone Point could have originated from other spawning grounds and were tidally tranported into the area.

Tidal export-import sampling was previously conducted at the mouth of the Niantic River (NUSCO 1987a) to estimate the net loss of winter flounder larvae from the river, and in 1988 five additional studies were conducted to verify earlier results. Sampling dates during 1988 were spaced over most of the larval winter flounder season to collect various developmental stages and size-classes. On March 24, a majority of the larvae collected were Stages 1 and 2 (89%); Stage 3 was
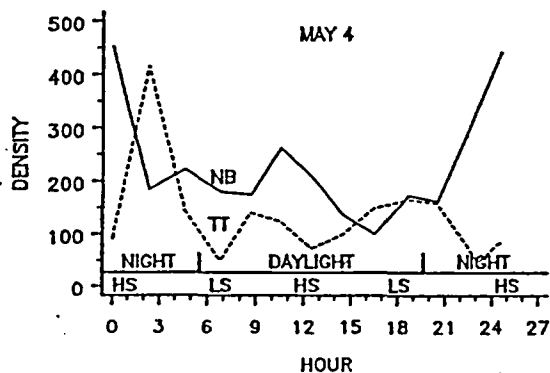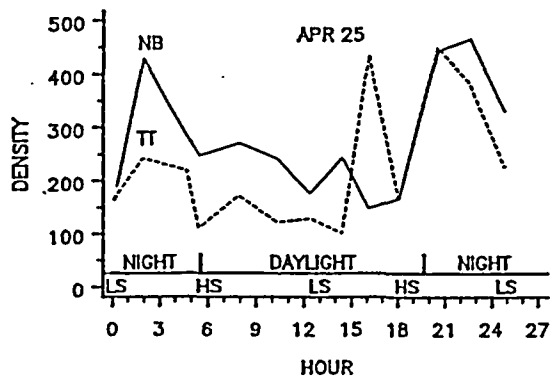
Fig. 16. Larval winter flounder densities (number per 500 m³) during two 24-h sampling periods at stations NB and TT in 1988 including the periods when night and daylight samples were collected and the time of low slack (LS) and high slack (HS) tides. Hours is the time from the start of sampling on each date.

Fig. 17. Percent occurrence by each developmental stage and 1-mm size-class of winter flounder larvae collected at the mouth of the Niantic River during ebb (E) and flood (F) tidal stages in 1988.

dominant on April 18 (68%) and May 2 (92%); and Stages 3 and 4 dominated on May 10 (92%) and May 17 (100%). For the five dates, the mean length ranged from 3.3 mm on March 24 to 6.8 mm on May 17. Examination of the percentage of each developmental stage for the combined data from all five dates showed that larvae of Stages 1 and 2 were more abundant during ebb tides and those of Stages 3 and 4 during flood tides (Fig. 17). Also, an examination by size-class showed that larvae 5 mm and smaller were more abundant during an ebb tide, where as, larvae 6

mm large were more abundant during a flood tide. Similar results were found in previous studies, except that the 5-mm size-class was more abundant during flood tide (NUSCO 1987a).

To determine if velocity measurements were comparable between ebb and flood tides, separate quadratic polynomial equations were fit to the hourly velocity measurements combined from each of the five ebb and flood tides sampled. Good fits were obtained with R^2 values exceeding 0.95 for both equations. The mean ebb duration was 6.7 h and flood duration was 5.7 h. The area under the curve for the flood tide (307.8) was smaller than for the ebb tide (414.4), indicating that flood velocities were low due to sampling location. To make ebb and flood velocities comparable, the flood velocities were estimated using a technique presented in NUSCO (1986a). These results were similar to those from previous studies

(NUSCO 1987a). The calculations of net larval exchange, which follow, were based on actual ebb current velocities and the adjusted flood current velocities.

Using data combined from the five sampling dates, net tidal exchange was estimated for each 1-mm size-class. The estimates were obtained by averaging the number of larvae per 500 m^3 of each size-class during each hourly sample for the five sampling dates. The average was multiplied by the estimated water velocity at the time of the hourly collection. This density-velocity adjustment accounted for changes in discharge volume during the tidal cycle. Because larvae collected during an ebb tide represented a loss from the river, the density-velocity value was made negative. A harmonic regression equation using a 12.4-h tidal cycle (the average duration of the five tides sampled) was fit to density-velocity values. The area under the curve for each tidal stage was estimated by numerical integration of the regression equation using 5-min increments. Net tidal exchange was expressed as the percent return of a size-class on a flood tide compared to loss on an ebb tide (Table 10). The harmonic regressions could not be satisfactorily fit to the 2- and 8-mm size-class data since the model sums of squares did not account for a significant (p = 0.05) amount of the total corrected sums of squares. The results showed a net export of 5 mm and smaller size-classes and a net import of 6 mm and larger size-classes. These results were similar to those of previous studies (NUSCO 1987a), except that there was a net loss of the 5-mm size-class in

1988, but a net gain (percent return of 131.8) in the earlier studies. This difference suggested that the 5-mm size-class was the size at which the transition from net loss to gain occurred and their relative abundance in relation to tidal stage may vary annually.

This change from a net loss of larvae from the river to a net gain as they get older has been attributed in the past to larval behavior with vertical migration in the water column as a retention mechanism (NUSCO 1987a). This behavior appears to become evident at the time of fin ray development, which allowed for better locomotion ability. Other researchers have also reported vertical migrations in early life history stages of fish. Diel movements of larval yellowtail flounder (*Limanda ferruginea*) were found to increase with larval size (Smith et al. 1978). Atlantic herring (*Clupea harengus*) larvae synchronize vertical migration with flood tides to minimize seaward transport (Fortier and Leggett 1983). Post-larval spot (*Leiostomus xanthurus*), Atlantic croaker (*Micropogonias undulatus*), and *Paralichthys* spp. flounders use vertical migration in response to tides as a retention mechanism (Weinstein et al. 1980). Larval North Sea plaice demonstrated selective horizontal transport by swimming up from the bottom during flood tides and remaining near the bottom during ebb tides (Rijnsdorp et al. 1985). Vertical migration by larval winter flounder in relation to tidal stage was a plausible explanation for the results of the export-import studies. However, an alternative hypothesis was that during the latter portion of the larval season large numbers of winter flounder larvae were tidally transported into Niantic Bay from other spawning areas and the larval density in the bay exceeded that of the river. Therefore, more larvae were present during a flood tide than an ebb. Several research projects are presently underway to examine this alternative hypothesis.

TABLE 10. Estimated percent return of larval winter flounder on a flood tide that were flushed from the river on an ebb tide presented by size-class with R^2 values of the harmonic regression models.

Size class (mm)	Percent return	R^2 of model
3	27.0	0.60
4	28.0	0.61
5	55.0	0.70
6	164.2	0.60
7	314.9	0.78

Growth and development

Examination of the length-frequency distribution of larvae collected in 1988 showed a separation between the first three developmental stages

FINAL REPORT

Larval Winter Flounder Stock Identification Using

Microelements: Year 2001 Studies

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February 7, 2002

EXECUTIVE SUMMARY

The goal of this project was to assess the degree to which winter flounder larvae are entrained from major local sources into the Millstone Nuclear Power Station (MNPS). The strategy involved the use and further development of a new multi-elemental tracer method using individual winter flounder larvae and established statistical analysis and neural network techniques. The emphasis was on multi-elemental analysis of individual larvae as this was of primary importance in tracking the origin of larvae from local sources into the Millstone Plant. Winter flounder larvae (primarily stage 2) were collected during March-April, 2001, from the Niantic and Thames Rivers, off Westbrook, and Plum Bank (Old Saybrook). Winter flounder larvae (stage 2-4) entrained into the Millstone Plant were also collected and analyzed from mid-April to mid-May. Samples were digested in a cleanroom laboratory and analyzed using inductively coupled plasma mass spectrometry (ICP-MS). A total of 247 individual winter larvae (stages 1-4) and 10 blanks were analyzed for 11 elements (2827 data points). Statistical analysis of these multi-elemental data using multivariate techniques and a three-layer neural network classifier indicate that a relatively small percentage (~10-20%) of winter flounder larvae entrained by the MNPS originate from the Niantic River. Results from this study further suggest that the use of microelements is a promising new tool for stock identification of the early life history stages of winter flounder and possibly other species, particularly when combined with independent techniques such as microsatellite DNA analysis.

INTRODUCTION

The population of the winter flounder local to the Niantic River is potentially affected by the operation of the Millstone Nuclear Power Station (MNPS), mainly by the entrainment of larvae through the cooling-water systems of the operating units (NUEL Annual Report, 1997). Since 1976, there have been extensive studies of the life history and population dynamics of this important sport and commercial species. It is now clear that a key goal is to assign individual entrained larvae to one of several stocks of larval winter flounder in this region. This requires a unique tracer, or "fingerprint", of the individual larva.

A recent report entitled "Stock Identification Research Directions for the Northeast Fisheries Science Center" by the NEFSC Stock Identification Working Group (January 10, 2000) provides an excellent summary of current research needs and some practical applications related to marine stock identification problems. An important objective in applied fishery management of some species is the ability to assign individuals to one of several of stocks in a given region. Substantial effort has already been focused on microsatellite DNA analysis, and the potential of DNA marker analysis is recognized. However, we suggest that the approach described herein also provides a unique and practical method for identifying source(s) of early life history stages of species, such as the winter flounder, which return with high fidelity to riverine-estuarine spawning areas that retain their early life history stages.

The approach conducted in this study was to use trace elements incorporated in whole winter flounder larvae as a

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tracer of the local environmental conditions, which were assumed to be unique for each spawning stock. The use of microelemental data to delineate fish stocks has been increasingly utilized via analysis of otoliths using highly sensitive laser ablation inductively coupled plasma mass spectrometry (e.g., Secor et al., 1995; Thresher et al. 1999). The key advantage in analyzing whole larvae, however, is that it is not necessary to remove a specific tissue component, such as the otolith, which adds significantly to the time involved in sample processing. Implicit in the whole larval analysis approach used in this study is that the trace element(s) are recorded in the larvae tissue, and we suggest that this is most likely within the larval otolith and other boney tissues.

This project expands on the initial studies conducted in 2000 (Moran, 2000; Saila and Lorda, 2000). Our whole larvae analysis technique and established statistical and neural network based classification techniques were used to assess the degree to which winter flounder larvae were entrained into the MNPS from major local sources.

The initial studies conducted by the PI, with assistance from Drs. Saila and Lorda, suggested that a relatively small percentage (<20%) of winter flounder larvae entrained by the MNPS originate from the Niantic River (Moran, 2000; Saila and Lorda, 2000). The details of the statistical and neural network methodologies used are similar to those proposed by Saila (1998). Based on our initial preliminary results, we suggested that this was a successful first-step in the development of a capability to determine a wide range of trace elements in individual winter flounder larvae.

In this report are the results from a more detailed study, conducted in 2001, and its application to the problem of larval entrainment by the MNPS. Results from this study further suggest that the use of microelemental analysis of whole larvae, rather than the otoliths, is a promising tool for stock identification of the early life history stages of winter flounder and other species, particularly when combined with independent techniques such as microsatellite DNA data analysis.

OBJECTIVES

The overreaching objective of this project was to further develop the use of a new technique to assess the degree to which early life history stages of winter flounder larvae are entrained into the MNPS using a combination of a new multi-elemental tracer approach and established statistical and neural network classification techniques. This multi-elemental approach is a first attempt at developing an empirical chemical tracer technique to assign individual larvae to one of several stocks. The details of the statistical and neural network methodologies to be applied in this proposal are very similar to those proposed by Saila (1998).

A total of 247 winter flounder larvae samples were collected from March-May, 2001, in conjunction with the ongoing Millstone Environmental Laboratory sampling operations. Samples were analyzed for a suite of trace elements by inductively coupled plasma mass spectrometry (ICP-MS). These data were then forwarded to Drs. Lorda and Saila for statistical analysis and interpretation. This work provides complementary information to independent stock

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identification techniques, specifically the use of microsatellite DNA analysis on larval samples being conducted concurrently by Dr. J. Crivello at the University of Connecticut.

The following provides a more detailed description of the previous work and current research strategy, including procedures used for sample collection and analysis, followed by results, data analysis, and conclusions.

PROPOSED WORK

A proposal was submitted to the MNPS in January, 2001, which outlined the following work to be completed. The proposed work was to utilize the recently developed multi-elemental analytical and statistical methods (Moran, 2000; Saila and Lorda, 2000) for stock identification of the early life history stages of winter flounder and other species. It was proposed that the application of this technique could be used to provide evidence regarding the sources of larval entrained at the MNPS. This involved collection of samples of winter flounder larvae from the Niantic and Thames Rivers, and west of the Connecticut River off Westbrook, and Plum Bank (Old Saybrook), and entrained larvae from MNPS.

The primary purpose of the Year 2000 study was to develop and assess the feasibility of this technique as a tool for stock identification. In order to fully develop this method, we proposed the following work for 2001 to improve confidence in the use of these multi-elemental and data analysis techniques.

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1)   Increase Sample-Size: The larval sample-size collected
during the Year 2000 study ranged from approximately 10-20
larvae per river (Moran, 2000). Moreover, the least number
of larvae were collected from the primary river, the Niantic
River.   Furthermore, only 5-10 entrained larvae were
analyzed for each collection period.   We proposed that a
total of approximately 150 larvae from the three rivers
sampled in this work be obtained in order to better
establish the initial conditions ("training sets") required
for data analysis. After discussions with Millstone
personnel, we further proposed that a sample size of
approximately 100-150 individual entrained larvae be
collected over several weeks in spring 2001 in order to
provide a more robust statistical analysis of these data.
Based on these recommendations, the total number of samples
to be analyzed increased from approximately 100 larvae in
Year 2000 to 247 larvae in Year 2001. .

2) Specific Elements: A primary objective of the Year 2000
study was to assess the feasibility of this multi-elemental
technique. For this reason, we analyzed each larvae for 30
elements. It became apparent that a smaller number of
elements may provide sufficient information to conduct a
statistically robust stock identification. Specifically,
based on results contained in the 2000 Final Report (Moran,
2000; Saila and Lorda, 2000), we proposed to focus on a
number of key elements (Co, Ni, Cu, Mo, Ba, Ce, Nd, Sm, Gd,
Au, and Pb), shown to provide the best discrimination in the
Year 2000 study.

Details of the proposed work, including sample collection,
sample processing, elemental analysis and statistical

analysis, have been documented (Moran, 2000; Saila and Lorda, 2000) and are summarized below.

SAMPLE COLLECTION

The proposed work involved collection of a total of 247 samples (105 population samples and 142 entrainment samples) from the aforementioned locations early March 2001 to mid-May, 2001. Samples collected were winter flounder larvae, of which fresh larvae (stage 1 and 2) were targeted in the spring.

At all times, samples were collected using clean techniques to avoid contamination from external sources. Samples were collected using clean techniques to avoid contamination from external sources. Source area larval samples were collected using a 60-cm bongo sampler, towed 6-8 minutes near the bottom. Contents of both replicates were rinsed with seawater into a 5-gallon plastic bucket.

A sub-sample (ca. 1 L) was placed in a plexiglass sorting tray onboard. Individuals were sorted and removed using a plastic pipette and transferred into a spotting glass containing de-ionized water. Individual larvae were separated from zooplankton using a plastic pipette and transferred to a final spot glass containing deionized Milli-Q water. Individual larvae were then transferred using teflon forceps to an acid-cleaned vial (ca. 2 mL), capped, and stored frozen until further processing in the cleanroom laboratory. Entrainment samples were taken at the power plant discharges using methods outlined in NUSCO (1997). Laboratory processing was the same as for field collected larvae.

SAMPLE PROCESSING

Samples were thawed and processed using a total microwave digestion technique, which was adapted from a method originally developed in the PI's laboratory for elemental analysis of marine particulate matter and sediment (Pike, 1998; Pike and Moran, 2001). Samples were processed in a clean room laboratory to reduce possible sample contamination from external sources (e.g., dust). Individual larvae were transferred from sample collection vials to a teflon bomb using an acid-cleaned pipette. Collection vials were rinsed with 2% ultrapure (sub-boiling distilled) HNO_3. Approximately 1 mL of concentrated HNO_3 and HF was added to each sample in the bomb and then digested in a microwave oven at low heat for 45 seconds. Digested samples were then subjected to mild heat overnight to ensure total digestion of the larvae. The final digested solutions were clear and devoid of any visible particle matter, indicating total digestion of the sample.

Samples were then evaporated to dryness in a clean hood and further evaporated two more times using 2% ultrapure HNO_3. Samples in 2% HNO_3 were transferred to preweighed, acid-cleaned vials and then weighed. Procedural blanks were prepared using the same digestion procedures as for the larval sample analyses.

SAMPLE ANALYSIS

A key technical advantage of the project involved the use of inductively coupled plasma mass spectrometry (ICP-MS). The rapid, multi-element capability, and high sensitivity of ICP-MS provided a powerful method for quantifying a wide

range of trace metals in the larval samples. Digested samples were analyzed for simultaneous determination of Co, Ni, Cu, Mo, Ba, Ce, Nd, Sm, Gd, Au, and Pb using a VG PlasmaQuad ExCell ICPMS. Matrix matched, multi-element standards were prepared using SPEX high purity standards. Instrument drift was monitored throughout the analysis. Quality control is based on analysis of certified reference materials (MESS-2, MESS-4, BCSS, PACS) (Pike, 1998; Pike and Moran, 2001).

Data reduction was conducted off-line. Linear regression calibration curves were prepared for each element and applied to all samples analyzed. Elemental concentrations in larvae are reported as ng element per individual larva.

It is worth noting that an alternative and/or complementary method could be developed to analyze the otoliths of individual winter flounder larvae using LA-ICPMS. This technique has the advantages of the multi-elemental analysis afforded by ICP-MS plus the ability to ablate, with a high degree of precision and accuracy, solid surfaces using a high-powered laser. Indeed, the use of LA-ICPMS is receiving increased application to fishery source identification and it is increasingly being recognized as a viable method for identifying source(s) of early life history stages of a number of fish species (Secor et al., 1995; Thresher et al., 1999; Yoshinaga et al., 2000; Zdanowicz, 2001). The minimum target spot size is 5 µm, which is well within the approximate 50-100 µm size of a winter flounder larval otolith. The use of LA-ICPMS would also reduce sources of external contamination, as a solid surface is analyzed and would avoid the need for sample

digestion and preconcentration, which is prone to sample contamination.

RESULTS

Results from the elemental analysis of winter flounder larvae are listed in the attached Tables (Tables 1-10). A total of 247 individual winter larvae and 10 blanks were analyzed for 11 elements, resulting in a total of 2827 data points. In addition, results from the statistical analysis of these data, and a comparison with the 2001 microsatellite DNA entrainment results (Crivello, 2002, in preparation), are presented in Tables 11-13.

STATISTICAL ANALYSIS

Statistical and neural network based classification of winter flounder larvae

The microelemental data listed in Tables 1-10 were analyzed using statistical techniques and neural network methodologies for classification. The ultimate goal was to assign an individual winter flounder larva to one of a number of predetermined groups. This type of problem is termed classification, and it is important in many areas of science. A common classification tool, the linear discriminant function, has desirable properties and was explored initially (Saila, 1999). The linear discriminant function can be considered as a form of Bayes rule that applies when the measurement vector comes from a multivariate normal distribution when all groups are assumed to have identical covariance matrices. If the covariance matrices were not equal, this leads to a quadratic form of

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the discriminant function. The above approaches were also combined with an artificial intelligence tool; namely, a three-layer neural network classifier (NeuroShell Easy Classifier™).

The microelemental data sets from the four known larval sources (Tables 1-4) were consolidated into a single "training data set" and the sources Plum Bank (PB) and Westbrook (WB), geographically undistinguishable, were pooled together as a single WB location. Screening statistical analyses were conducted on this training data set prior to the final discrimination analysis. The screening process involved the following steps carried out with the SAS computer programs:

1) All data sets were first screened for negative concentrations (changed to zeroes) and for unusually large concentrations likely resulting from sample contamination. The latter were removed and replaced with the mean concentration of that particular microelement in the specific location where the abnormal data was found. In a final step prior to any subsequent analysis, the data were subjected to logarithmic transformation (i.e., $\log_e[x+1]$) to reduce the disparity of scales among the eleven microelements.

2) Linear correlation analyses were conducted to establish whether larval size was related to the concentrations of the eleven microelements; the results showed significant correlation for some large larvae. It was concluded that a few larvae larger than 6.5 mm should be excluded prior to the discrimination analysis (see below).

3) Multiple means comparisons of microelemental
   concentrations among the three sources were conducted for
   each of the eleven chemical elements in an attempt to
   select only elements with significant mean concentration
   differences. Since no significant differences among
   sources could be found for any of the microelements, it
   was decided to use all of them in all subsequent
   discrimination analyses.

Given that some of the data were clearly not meeting the
requirements of normality and homogeneity of variance for
standard parametric statistical analysis, only nonparametric
discriminant methods were applied. In the case of the
training data sets (i.e., from larvae of known sources), the
best discrimination results were obtained with a K-nearest-
neighbor method with a parameter K=2 chosen because it gave
the best cross-validated estimate of the error rate. This
same method with K=2 was subsequently used to identify
possible larval sources in the Entrainment data sets (Tables
5-10), for which actual sources were unknown. The screening
step #1 above was applied to the raw entrainment data prior
to the discrimination analysis. A second nonparametric
discriminant function based on a kernel method using kernel
density estimates with unequal bandwidth was applied to both
training and the entrainment data sets with no discernible
improvement of the discrimination results.

A totally different classification method applied to the
above data was based on a three-layer neural-network
classifier algorithm (NeuroShell Easy Classifier™) requiring
no assumptions for the data. This class of algorithms use
the information content in the data regardless of
distribution, scales, or units. The specific algorithm used

was from the computer program "Neuro-Shell Classifier" developed by Ward Systems Group, Inc., (www.wardsystems.com) widely used in medical and pharmaceutical research.

Results from the analysis of the training set data using a nonparametric discriminant function with K=2 (Table 11) indicated 76-81% of larvae matched the source regions. By comparison, analysis of the training set data using a three-layer neural network model indicated 84-93% classification sensitivity (Table 12). Thus, considering just the training set data, these classification results were satisfactory.

When applied to the entrained larvae samples (Table 13), the nonparametric discriminant function with K=2 suggests a classification of 4%, 0% and 66% to the Niantic and Thames Rivers and off Westbrook, respectively, and 30% to other sources not classified, based on the training set data provided. Using this classification model with kernal density estimates with equal bandwidth, the classification results were 14% Niantic, 5% Thames, 80% Westbrook, and 0% to other sources. The entrainment analysis results obtained using the neural network classifier ranged from 10 to 20% Niantic, 2 to 4% Thames, 33 to 61% Westbrook, and 15 to 55% to other sources, using a cut-off probability of 0.65 and 0.5, respectively. This compares with preliminary results of the neural network analysis of the 2001 microsatellite DNA data (Crivello, 2002, in preparation) of about 24% Niantic, 22% Thames, 35% Westbrook, and 19% to other sources (Table 13).

It is encouraging that the microelemental and microsatellite DNA results suggest a similar classification probability to the Niantic River; namely, 10-20%. Also, the 33%

classification to Westbrook (0.65 cut-off) and 15% to other sources (0.5 cutoff) is consistent with the microsatellite DNA results. However, using the neural network analysis classifier, the microelemental data suggests a relatively low percentage assigned to the Thames River compared to the microsatellite DNA data. This result is unusual, and apparently inconsistent with the known tidal circulation pattern in this region that is expected to deliver significant quantities of larvae from the Thames River to the Millstone Plant.

One possible explanation may be that the elemental composition of the larvae assigned to other sources may have been similar to the Thames River elemental composition, at least in 2001, thereby complicating the classification. Supporting this suggestion is the fact that elemental concentrations in the larvae in Year 2001 were extremely low, making it more difficult to distinguish between geographical sources. In this regard, examination of additional elemental data (above the 11 elements examined) from the larvae analyzed in 2001 may provide further information.

In addition to using a neural network for classifying individual winter flounder larvae into known groups, an effort was also made to utilize an unsupervised clustering method to estimate the optimum number of groups in a data set without any a priori information about the actual number of groups in the data. The purpose of this work was to be able to obtain a grouping from sample data without any other information. The specific fuzzy clustering method used in this study is described in Kaufmann and Rousseeuw (1990).

The fuzzy clustering method was applied to the training set for microelements, but no information on individual larval sources was provided. Fuzzy clustering was applied to these data under the assumption of 2,3,4,5 and 6 groups. It was observed that the clustering into 3 groups provided the best grouping, based on Dunn's partition coefficient. This confirmed that the unsupervised clustering method appeared to work in a satisfactory manner with training set data by providing a similar partitioning of the individuals. However, the attempt to separate the entrainment data set into groups was not successful. It appeared that these data were more variable to the extent that this particular clustering algorithm could not effectively distinguish groups.

Further efforts to use other unsupervised clustering methods were undertaken. An approach to fuzzy clustering first described by Marsili-Libelli (1989) was developed and tested. This uses a fuzzy partition algorithm, and fuzzy partition efficiency was measured by a normalized partition entropy. It has been indicated by the above author that the normalized partition entropy corresponds to a maximization of the likelihood that a given data set X may indeed contain C subsets with homogeneous features. Our program utilizes algorithm 1 of Marsili-Libelli and normalized partition entropy. It was applied to an entrainment sample which consisted of N = 113 observations with 11 variables per observation. It was found that the optimum number of clusters was three with an optimal entropy index of 0.0213576. The results from this unsupervised algorithm suggest that the entrainment data analyzed consists of three distinct groups of winter founder larvae. This corresponds with a familiar finding of three groups for the training

set. The results of this analysis for the entrainment samples suggest that these larvae also consist of three distinct groups, which are assumed to correspond to the training set samples. However, there is no valid procedure for comparing these results with the neural net predictions because the assignment methods differ. This analysis also indicated that only one individual larva of the 113 individuals was classified as unknown by virtue of a calculated possibility of membership in any group of less than the 0.65.

Relationship between microelemental composition and total length of winter flounder larvae

Total length measurements were made for a sample of the training set larvae prior to analysis of their whole body microelemental composition by ICPMS. The purpose was determine whether or not there is a significant functional relationship between size of sampled larvae and microelemental composition and, if a significant relationship was found, to provide advice as to how it should be treated.

Initially, a Pearson product moment correlation was calculated between larval size of all sampled larvae and a suite of the eleven elements analyzed. That is, all sampled sites were combined and correlated with each individual element. The results of this analysis indicated no statistically significant correlation for eight of the eleven elements. The three elements for which low (but statistically significant) correlation's were obtained in this manner were Cu, Mo and Pb.

A further careful analysis of the relation between size and quantities of these elements was then made. This was done using an automated curve fitting program which fitted a series of 105 single term functions with intercepts to the data regarding the above elements. This curve fitting procedure assured that the best fitting equation based on a r^2 and F statistic values was selected in each application. It was decided to test whether the initial correlations for the three elements listed as significant were affected by merging all samples prior to analysis. Therefore, separate analyses were conducted for establishing relations between size and composition for each sample site (namely Niantic River, Thames River, and Westbrook) versus each element (namely Cu, Mo, Pb).

The results of this work are summarized as follows. No significant relationship between size and concentration of any of the three elements was found for the Niantic River data. No significant relationship between the concentration of the three elements and larval size were found for the Thames River data. However, a significant functional relationship was found between Cu and size for the Westbrook sample data. This significant relationship was eliminated by truncating the individual larval size at 7 mm.

A one-way analysis of variance (ANOVA) clearly indicated that the average size of Westbrook larvae was greater than the Niantic or Thames River larvae. Similarly, it was found that a significant relation between Mo and size occurred when all Westbrook data were utilized. This relationship was eliminated by truncating the sample larvae at 6.5 mm. No significant relation between Pb concentration and size was found for Westbrook larvae. In summary, no significant

relation between larval size and microelemental concentration is expected if the size of the largest larvae used for classification using microelements can be limited as indicated above.

CONCLUSIONS

1) Based on a three-layer neural network analysis, a relatively small percentage (10-20%) of larvae entrained into the Millstone Power Station were from the Niantic River for the period analyzed in March-May, 2001.

2) The classification of 10-20% of entrained larvae to the Niantic River based on the microelemental data is in reasonable agreement with preliminary microsatellite DNA results (about 24%) for this sampling year.

3) Analysis of the microelemental data suggests a greater classification probability to Westbrook (Connecticut River area) and other (nonclassified) sources and a relatively smaller classification probability to the Thames River than determined when using the microsatellite DNA and a neural network classification model.

4) Results from an unsupervised fuzzy clustering algorithm (Marsili-Libelli, 1989) suggest that the entrainment data analyzed consists of three distinct groups of winter founder larvae. This corresponds with a familiar finding of three groups for the training set. Results of this analysis for the entrainment samples suggest that these larvae also consist of three distinct groups, which are assumed to correspond to the training set samples.

5) No significant relation between larval size and microelemental concentration is expected if the size of the largest larvae used for classification using microelements can be limited to <6.5 mm.

DATA PRESENTATIONS AND PUBLICATIONS

A preliminary presentation of this work was made at the 2001 Millstone Environmental Advisory Council Meeting, June 12-14, 2001. These results will be used to prepare a manuscript for submission to a peer-reviewed scientific journal.

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Table 12 - 2001 NeuroShell Easy Classifier™, training data.

Table 13 - 2001 micro-elemental and microsatellite DNA entrainment data analysis results summary.

Table 1 - 2001 Thames River Training Sets - Final (ng element per individual larvae).

Sample	L (mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
TR 1-1	5.0	0.1390	1.3444	0.9447	0.0251	0.0792	-0.0076	0.0162	0.0045	0.0090	0.0004	0.3026
TR 1-2	5.2	0.1194	1.7318	1.0334	0.0273	0.1552	-0.0503	0.0038	0.0024	0.0088	0.0009	0.2594
TR 1-3	4.5	0.1169	1.6668	1.7761	0.0313	0.0117	-0.0534	0.0038	0.0027	0.0088	0.0003	0.2460
TR 1-5	5.9	0.1008	1.4890	0.5281	0.0251	0.0377	-0.0488	0.0010	0.0013	0.0083	0.0004	0.2606
TR 1-8	5.2	0.1175	1.4890	1.6902	0.0280	0.0756	-0.0465	0.0040	0.0012	0.0084	0.0002	0.6621
TR 1-10	5.1	0.1254	1.5849	1.4081	0.0404	0.0939	-0.0453	0.0057	0.0022	0.0082	-0.0001	0.5360
TR 1-12	6.0	0.1040	1.4259	1.1633	0.0139	0.1864	-0.0424	0.0016	0.0033	0.0090	0.0003	0.4348
TR 1-13	6.3	0.1925	30.0135	4.2337	0.0247	0.1980	-0.0455	0.0026	0.0015	0.0081	0.0002	0.4572
TR 1-16	6.3	0.1187	1.4901	1.6802	0.0254	0.0625	-0.0129	0.0250	0.0064	0.0109	0.0002	0.4329
TR 2-1	3.7	0.1222	1.4376	1.4468	0.0394	0.0196	-0.0472	0.0028	0.0013	0.0074	0.0002	0.3562
TR 2-2	4.4	0.5298	1.8676	2.5503	0.0378	0.0688	-0.0539	0.0016	0.0014	0.0084	0.0001	0.3039
TR 2-8	4.2	0.0994	1.4815	0.8764	0.0281	-0.0600	-0.0382	0.0005	0.0010	0.0080	-0.0002	1.2616
TR 2-10	4.4	0.1046	1.5700	1.1101	0.0240	0.1251	-0.0166	0.0213	0.0056	0.0099	-0.0001	0.2636
TR 2-12	5.7	0.1124	1.3607	1.3206	0.0154	1.4766	-0.0335	0.0098	0.0028	0.0087	0.0002	0.5252
TR 2-13	4.0	0.0888	1.2825	0.4836	0.0245	0.3887	-0.0451	0.0015	0.0018	0.0070	0.0000	0.2748
TR 2-15	4.0	0.1043	1.3597	0.9913	0.0142	-0.0069	-0.0524	-0.0004	0.0006	0.0081	-0.0001	0.2617
TR 2-17	4.3	0.1296	1.8169	13.2825	0.0158	0.3319	-0.0611	0.0010	0.0019	0.0094	0.0001	0.2592
TR 2-19	3.8	0.1237	1.7218	0.4951	0.0185	-0.0089	-0.0245	0.0190	0.0056	0.0115	0.0001	0.2222
TR 2-21	4.1	0.1011	1.4755	0.5293	0.0154	-0.0802	-0.0485	0.0010	0.0013	0.0075	0.0004	0.2862
TR 2-23	3.5	0.1332	1.7117	1.1399	0.0106	0.0038	-0.0487	0.0038	0.0005	0.0081	-0.0001	0.4555
TR 3-1	6.6	0.1390	1.9800	0.8324	0.0194	0.3472	-0.0565	0.0022	0.0024	0.0093	0.0008	0.3398
TR 3-2	4.0	0.4550	2.0086	4.3136	0.2797	50.6500	-0.0435	0.0031	0.0024	0.0074	0.0003	1.3052
TR 3-3	5.8	0.1376	1.7764	1.5069	0.0573	0.2197	-0.0095	0.0250	0.0070	0.0107	0.0013	0.4764
TR 3-5	6.0	0.1363	1.5680	2.2406	0.0387	0.3258	-0.0461	0.0027	0.0018	0.0082	0.0006	0.4796
TR 3-9	7.0	0.0935	1.3587	1.0608	0.0172	0.1572	-0.0406	0.0025	0.0015	0.0065	0.0000	0.3945
TR 3-10	5.9	0.1185	1.7867	1.2492	0.0151	0.1744	-0.0553	0.0039	0.0017	0.0089	0.0001	0.5276
TR 3-11	4.2	0.1189	1.8878	3.1296	0.0268	0.0671	-0.0192	0.0169	0.0063	0.0100	0.0000	0.8047
TR 3-12	6.8	0.1366	2.8612	128.3488	0.0201	0.0730	-0.0491	0.0038	0.0017	0.0080	-0.0001	6.2705
TR 3-13	7.2	0.1703	1.6959	2.3467	0.0462	0.1315	-0.0270	0.0043	0.0016	0.0077	0.0003	0.6571
TR 3-16	5.5	0.1389	1.6726	1.6060	0.0469	0.0993	-0.0565	0.0007	0.0011	0.0087	-0.0003	0.4323
TR 3-18	4.5	0.1075	1.5162	1.0140	0.0101	-0.0705	-0.0520	-0.0012	0.0007	0.0072	-0.0001	0.2675
TR 3-19	5.2	0.1200	1.5413	0.4695	0.0220	0.2289	0.1662	0.1033	0.0209	0.0143	-0.0002	0.2514
ave		0.1455	2.5305	5.8376	0.0339	1.7363	-0.0347	0.0092	0.0031	0.0088	0.0002	0.6334

Table 2 - 2001 Plum Bank Training Sets - Final (ng element per individual larvae).

Sample	L (mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
PB 1-1		0.0969	1.3033	0.7948	0.0135	-0.0342	-0.0388	0.0036	0.0014	0.0070	0.0002	0.2128
PB 1-2		0.1106	1.7347	1.1347	0.0171	-0.0709	-0.0467	0.0018	0.0012	0.0073	0.0000	0.2488
PB 1-3		0.1001	1.3078	1.0859	0.0159	-0.0663	-0.0449	0.0000	0.0008	0.0069	0.0006	0.1959
PB 1-5	4.5	0.0986	1.8605	1.9064	0.0891	0.0768	-0.0152	0.0177	0.0044	0.0091	0.0001	0.3767

Table 3 - 2001 Niantic River Training Sets - Final (ng element per individual larvae).

Sample	L (mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
NR 1-B		0.2451	1.3744	2.5457	0.0153	0.0423	-0.0439	0.0016	0.0015	0.0066	0.0000	0.3735
NR 1-1		0.0684	1.1326	1.1570	0.0094	0.0837	-0.0447	0.0014	0.0011	0.0065	0.0001	0.4091
NR 1-2		0.0835	1.2046	1.5271	0.2002	0.4942	-0.0428	0.0002	0.0010	0.0058	0.0000	0.2970
NR 1-3		0.0855	1.3475	2.1260	0.0123	0.0151	-0.0392	0.0022	0.0017	0.0060	0.0000	0.4028
NR 1-4		0.0722	1.0878	1.4269	0.0222	0.0448	-0.0138	0.0190	0.0048	0.0077	0.0001	0.8208
NR 1-5		0.0821	1.1555	1.0586	0.0170	-0.0289	-0.0411	0.0028	0.0008	0.0062	0.0001	0.2157
NR 1-6		0.0582	1.0601	0.3457	0.0106	-0.0335	-0.0432	0.0015	0.0011	0.0065	0.0000	0.2671
NR 1-7		0.0723	1.1010	1.4235	0.0105	0.0680	-0.0390	0.0006	0.0015	0.0058	-0.0001	0.3205
NR 1-8(1)		0.0686	1.1710	0.3713	0.0110	-0.0737	-0.0508	-0.0001	0.0007	0.0070	0.0003	0.1572
NR 1-8(2)		0.5696	1.6274	0.8075	0.0419	0.1356	0.0059	0.0285	0.0087	0.0096	0.0004	1.2417
NR 1-9		0.0775	1.1846	0.3127	0.0099	0.0747	-0.0252	0.0104	0.0030	0.0066	-0.0001	0.4807
NR 1-10		0.0748	8.2519	0.7264	0.0118	-0.0481	-0.0367	0.0054	0.0014	0.0060	0.0001	0.3160
NR 1-11		0.0920	1.7764	0.4936	0.0143	0.0351	-0.0378	0.0337	0.0062	0.0092	-0.0001	0.1896
NR 1-12		0.0626	1.1582	0.6173	0.0168	-0.0100	-0.0377	0.0040	0.0022	0.0067	0.0017	0.2071
NR 1-13		0.0804	1.3843	2.1719	0.0201	0.2380	0.0341	0.1900	0.0380	0.0198	0.0000	0.2819
NR 2-1	3.3	0.0537	1.3016	1.2721	0.0267	0.2610	-0.0274	0.0056	0.0010	0.0049	0.0003	0.4332
NR 2-3	3.4	0.0700	0.9912	0.5777	0.0178	-0.0598	-0.0473	0.0011	0.0011	0.0064	0.0000	0.2284
NR 2-4	3.6	0.0695	1.2572	0.8089	0.0291	0.0153	-0.0432	0.0034	0.0017	0.0074	0.0000	0.5500
NR 2-5	3.7	0.0880	1.1924	0.5219	0.0622	0.2897	-0.0215	0.0157	0.0052	0.0083	0.0000	0.3159
NR 2-6	3.4	0.0609	1.1137	0.7206	0.0244	-0.0357	-0.0462	0.0015	0.0018	0.0075	-0.0001	0.2097
NR 2-7	4.1	0.0624	1.4511	1.4067	0.1341	0.1412	-0.0317	0.0053	0.0023	0.0061	0.0002	0.6724
NR 2-11	3.1	0.0541	0.9366	0.8568	0.0146	0.0262	-0.0308	0.0078	0.0024	0.0057	0.0002	0.2987
NR 2-13	3.8	0.0622	0.9974	0.3796	0.0126	-0.0480	-0.0414	0.0044	0.0012	0.0061	0.0000	0.2164
NR 2-15	3.9	0.0816	1.0848	1.2730	0.0157	0.0688	-0.0179	0.0123	0.0042	0.0070	0.0000	0.1950
NR 2-17	3.9	0.1158	1.0729	0.7285	0.0173	0.1301	-0.0222	0.0042	0.0019	0.0045	0.0001	0.4845
NR 2-18	3.6	0.1068	26.4528	0.7761	0.0152	0.0352	-0.0392	0.0021	0.0011	0.0065	0.0000	0.3852
NR 2-19	3.9	0.0960	2.2577	0.6658	0.0142	0.0956	-0.0468	0.0002	0.0017	0.0067	0.0001	0.2504
NR 2-21	3.5	0.0962	1.9687	4.7716	0.0160	-0.0044	-0.0485	0.0006	0.0011	0.0073	0.0001	0.3020
NR 2-22	4.1	0.1075	1.8808	0.9179	0.0158	0.2420	-0.0179	0.0143	0.0040	0.0085	0.0001	0.1993
NR 2-23	3.8	0.1128	1.8889	0.7257	0.0141	0.9932	-0.0480	-0.0012	0.0010	0.0075	-0.0002	0.1381
NR 3-1	4.2	0.1047	1.9586	0.9304	0.0216	0.0730	-0.0409	0.0003	0.0010	0.0061	0.0001	0.3053
NR 3-2	3.9	0.0929	1.6320	0.5567	0.0691	0.1065	-0.0101	0.0207	0.0057	0.0077	0.0090	0.4151
NR 3-3	3.9	0.1489	2.9343	0.9257	0.0378	-0.0446	-0.0451	0.0012	0.0004	0.0067	0.0005	3.7244
NR 3-8	6	0.1679	7.7198	1.7419	0.2241	-0.3192	-0.0218	0.0141	0.0030	0.0073	0.0001	1.2400

NR 3-9	5.2	0.1052	1.6908	0.6592	0.0158	0.1155	-0.0428	0.0005	0.0013	0.0063	0.0003	0.1523
NR 3-10	3.3	0.1629	2.6263	2.6247	0.0671	2.1659	0.0290	0.0190	0.0047	0.0078	0.0913	1.6093
NR 3-11	3.1	0.1349	3.1007	1.4608	0.0269	-0.0196	-0.0373	0.0014	0.0017	0.0066	0.0077	0.3259
NR 3-12	3.3	0.1070	2.3502	0.8718	0.0259	0.1773	-0.0149	0.0149	0.0032	0.0071	0.0004	0.5127
NR 3-15	3.9	0.2005	3.9186	1.0098	0.0170	0.0362	-0.0569	0.0020	0.0027	0.0092	0.0001	0.3463
NR 3-16	4	0.0780	0.9344	1.8608	0.0139	-0.0257	-0.0357	0.0058	0.0021	0.0085	0.0006	0.2517
NR 3-19	3.8	0.1864	3.0805	1.8895	0.0440	0.0509	-0.0102	0.0145	0.0031	0.0081	0.0324	0.3367
NR 3-22	3.2	0.1213	1.7552	0.2791	0.0164	-0.0484	-0.0213	0.0067	0.0007	0.0064	0.0006	0.1718
ave		0.1105	2.5135	1.1506	0.0341	0.1451	-0.0308	0.0114	0.0032	0.0072	0.0035	0.4822

Table 4 - 2001 Westbrook Training Sets - Final (ng element per individual larvae).

Sample	L (mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
WB 1-1		0.1696	1.7655	5.2662	0.0301	0.1671	-0.0359	0.0036	0.0008	0.0067	0.0005	0.4482
WB 1-2	3.8	0.1496	1.6548	2.0446	0.0230	0.0242	-0.0514	0.0017	0.0008	0.0078	0.0001	0.3177
WB 1-3		0.1204	1.7602	2.9598	0.0302	0.0568	-0.0483	0.0051	0.0016	0.0085	0.0001	0.4284
WB 1-4	4.7	0.1293	1.7936	2.8666	0.0251	-0.0351	-0.0538	0.0025	0.0015	0.0085	-0.0003	0.3620
WB 1-6		0.1318	1.7587	1.3767	0.0776	2.4710	-0.0217	0.0193	0.0075	0.0095	0.0001	0.7674
WB 1-9	5.1	0.1077	1.5767	1.5222	0.0342	0.0847	-0.0501	0.0037	0.0017	0.0083	0.0009	0.4301
WB 1-10	4.9	0.0981	1.4346	1.9411	0.0459	0.0711	-0.0559	0.0015	0.0014	0.0083	0.0002	0.3315
WB 1-12		0.1326	1.5012	4.4052	0.0519	0.4078	-0.0270	0.0297	0.0092	0.0105	0.0008	1.3719
WB 1-14		0.0918	1.1760	1.0631	0.0233	0.1584	-0.0198	0.0213	0.0054	0.0092	0.0003	0.3007
WB 1-15		0.1057	1.4736	1.9432	0.0503	0.0212	-0.0474	0.0032	0.0024	0.0075	0.0004	0.6330
WB 1-16		0.0932	1.3268	0.7492	0.0225	-0.0894	-0.0584	-0.0014	0.0007	0.0077	0.0012	0.1566
WB 1-18		0.0881	1.2260	1.3028	0.0244	-0.0917	-0.0570	-0.0003	0.0014	0.0075	0.0003	0.6037
WB-1-19		0.1054	1.3213	1.2277	0.0302	0.0900	-0.0146	0.0204	0.0045	0.0106	0.0009	0.4304
WB 2-1	5.1	0.1045	1.4094	0.9638	0.0337	0.1724	-0.0146	0.0280	0.0053	0.0094	0.0004	0.4568
WB 2-2	4.6	0.0902	1.2779	5.4085	0.0186	-0.0030	-0.0519	0.0011	0.0009	0.0070	0.0005	1.5512
WB 2-3	8.5	0.0997	1.3644	1.6147	1.2158	0.0121	-0.0532	0.0030	0.0014	0.0088	0.0007	0.3840
WB 2-4	3.3	0.9785	5.6027	33.8724	0.0364	0.7140	-0.0376	0.0110	0.0020	0.0082	0.0011	2.8002
WB 2-5	4	0.0999	1.4073	1.3001	0.0221	0.0442	-0.0224	0.0160	0.0057	0.0099	-0.0001	0.9193
WB 2-6	7	0.0812	1.2998	0.9833	0.0217	-0.0800	-0.0473	0.0025	0.0011	0.0084	0.0008	0.2704
WB 2-11	4.1	0.0843	1.4457	0.8778	0.0115	0.2635	-0.0454	0.0030	0.0020	0.0070	-0.0001	0.3814
WB 2-12	5.3	0.0908	1.6721	1.0465	0.0207	-0.0176	-0.0561	0.0017	0.0016	0.0077	0.0000	1.2668
WB 2-17	6.4	0.0823	1.2270	0.8963	0.0235	0.0501	-0.0195	0.0159	0.0069	0.0085	0.0010	0.2891
WB 2-19	3.8	0.0768	1.5966	0.8836	0.0171	-0.0040	-0.0380	0.0044	0.0028	0.0066	0.0002	0.4124
WB 2-20	4	0.1017	1.4189	0.5173	0.0117	0.1056	-0.0605	0.0004	0.0013	0.0085	-0.0001	1.3464
WB 2-22	3.7	0.0841	1.5088	1.0476	0.0240	0.0151	-0.0501	0.0040	0.0009	0.0084	0.0001	0.2875
WB-2-24	3.2	0.0822	1.4212	1.0833	0.0213	0.0090	-0.0259	0.0158	0.0060	0.0092	0.0001	0.3558
ave.		0.1377	1.6316	3.04475	0.0749	0.1776	-0.04093	0.0083	0.003	0.008	0.0004	0.6655

Table 5 - 2001 Blanks - Final (ng)

Sample	L (mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
WB 1-8	blank	0.1181	1.6484	1.6455	0.0385	0.0011	-0.0548	0.0010	0.0021	0.0079	0.0001	0.2384
WB 1-17	blank	0.1057	1.3449	0.7993	0.0786	-0.0699	-0.0614	-0.0012	0.0009	0.0083	-0.0003	0.1887
WB 2-7	blank	0.1166	1.2893	1.0701	0.0087	-0.0112	-0.0574	-0.0016	0.0010	0.0081	0.0000	0.1967
WB 2-21	blank	0.0737	1.2292	0.5899	0.0147	-0.1020	-0.0517	0.0015	0.0011	0.0076	-0.0002	0.4116
TR 1-7	blank	0.1063	1.4900	0.7528	0.0447	0.2625	-0.0121	0.0158	0.0052	0.0091	0.0001	0.2702
TR 2-7	blank	0.1258	1.7212	1.4245	0.0216	0.0986	-0.0465	0.0066	0.0018	0.0085	-0.0003	0.4819
TR 3-7	blank	0.1439	1.9334	2.1143	0.0185	0.0933	-0.0385	0.0016	0.0022	0.0090	-0.0001	0.7061
PB 1-4	blank	0.0961	1.3236	0.8949	0.0137	-0.0659	-0.0498	0.0029	0.0015	0.0077	0.0003	0.2849
NR 2-B	blank	0.1007	1.5774	3.9013	0.0467	0.4748	-0.0254	0.0094	0.0022	0.0069	0.0002	1.5361
NR 3-7	blank	0.1839	2.9743	1.7334	0.0477	0.3999	-0.0190	0.0207	0.0072	0.0097	-0.0001	0.3542

Table 6 - Entrainment Data Set#1 2001 (ng element per individual larvae)

Sample	L(mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
E-1-1	5.1	0.15819	2.05172	2.22951	0.03067	0.21725	0.04160	0.02386	0.00378	0.00373	0.00107	0.44537
E-1-3	5.5	0.14184	2.19115	1.11147	0.03585	0.09050	0.00791	0.00773	0.00042	0.00212	0.00100	0.24883
E-1-5	7.9	0.17157	2.44778	1.69573	0.04198	0.28152	0.00726	0.01174	0.00163	0.00304	0.00063	0.62617
E-1-7	5.9	0.16942	2.27804	2.28706	0.05831	0.19938	0.00434	0.00752	0.00122	0.00393	0.00102	1.00446
E-1-10	6.1	0.16976	2.06295	1.44320	0.03581	0.21933	0.00274	0.00887	0.00263	0.00504	0.00080	0.39518
E-1-12	6.7	0.17582	2.61985	2.16413	0.03622	0.33308	0.00039	0.01430	0.00285	0.00219	0.00134	0.61087
E-1-14	7.4	0.18167	2.16159	1.93820	0.02921	0.15308	0.03893	0.05018	0.01042	0.01057	0.00110	4.05547
E-1-15	6.3	0.15104	1.91826	1.25366	0.03785	0.25804	0.00517	0.00862	0.00095	0.00109	0.00091	0.32369
E-1-17	-	0.16871	2.09755	1.42483	0.03598	0.15433	0.05498	0.04646	0.00733	0.01166	0.00184	0.23743
E-1-18	7.6	0.21899	2.69575	3.84433	0.07893	1.33961	0.04021	0.03475	0.00684	0.00655	0.00155	1.61172
E-1-19	6.3	0.20837	2.24818	1.79228	0.09210	0.90045	0.02886	0.02736	0.00619	0.00592	0.00093	0.78210
E-1-21	6.2	0.16364	2.30369	1.86131	0.04335	0.26847	-0.00371	0.00969	0.00156	0.00114	0.00145	0.90566
E-1-22	4.6	0.16893	2.00815	3.01516	0.03363	0.18447	-0.00300	0.00747	0.00110	0.00215	0.00112	0.55090
E-1-23	7.5	0.26252	3.19659	32.68435	0.10094	5.46504	0.12400	0.07072	0.01221	0.01376	0.00500	12.42337
E-1-26	7	0.59761	2.36908	1.98863	0.06344	0.31054	0.02024	0.00797	0.00048	0.00173	0.00195	0.97689
E-1-27	6.2	0.19032	2.20163	2.14498	0.06615	0.27883	0.00593	0.02533	0.00211	0.00390	0.00140	0.60230
E-1-28	7.5	0.20004	2.05886	2.30398	0.07998	1.03620	1.09828	0.04954	0.00303	0.02274	0.00196	0.65391
E-1-30	5.7	0.14392	1.87516	1.24434	0.04120	0.11899	-0.00554	0.00177	-0.00027	-0.00026	0.00076	0.31901
E-1-31	7.3	0.19436	2.24819	3.69603	0.06847	0.39621	0.03186	0.02872	0.00597	0.00660	0.00102	0.57131
E-1-32	7.3	0.18214	2.33535	2.22416	0.05160	0.34582	0.00293	0.06186	0.00173	0.00250	0.00173	0,95553
E-1-33	6.8	0.15728	2.00790	2.17079	0.06215	0.24696	-0.00406	0.00442	0.00074	0.00088	0.00163	0.64578

Table 7 - Entrainment Data Set #2 2001 (ng element per Individual larvae)

Sample	L (mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
E-2-1	5	0.19662	2.49865	3.10124	0.14564	0.39073	0.05254	0.04107	0.00658	0.00647	0.00213	0.62525
E-2-4	4	0.15594	1.96560	2.46015	0.04024	0.14179	-0.00187	0.00708	0.00077	0.00157	0.00071	0.35947
E-2-5	5	0.15187	1.71863	1.82327	0.05337	0.21441	-0.00145	0.01035	0.00074	-0.00017	0.00173	0.54007
E-2-8	5	0.20701	2.06539	1.63504	0.08188	0.77767	0.05521	0.03544	0.00797	0.00748	0.00163	0.53261
E-2-9	4.9	0.15932	1.95531	1.91838	0.04339	0.24928	0.00672	0.00739	0.00122	0.00090	0.00083	0.99301
E-2-10	5.9	0.18322	1.96448	2.95921	0.04406	0.42909	0.01988	0.01897	0.00473	0.00364	0.00075	0.59582
E-2-11	6.6	0.19170	1.96245	2.63159	0.06313	1.08401	0.05681	0.03945	0.00746	0.00432	0.00130	0.64059
E-2-13	3.9	0.84429	2.54818	4.98538	0.11151	4.38614	0.00116	0.00924	0.00138	0.00112	0.00637	2.26569
E-2-14	4.4	0.34108	2.03798	7.18580	0.14350	0.42944	0.03774	0.03096	0.00723	0.00520	0.00242	6.86795
E-2-16	3.9	0.17833	1.91206	1.55564	0.18275	0.36140	-0.00582	0.00647	0.00111	0.00083	0.00120	0.60285
E-2-19	4.8	0.21292	1.83034	1.08226	0.04815	0.59197	-0.00541	0.00319	0.00032	0.00026	0.00052	0.59429
E-2-22	4.3	0.13381	1.68634	0.79148	0.03666	0.10711	-0.00479	0.00638	-0.00030	0.00025	0.00090	0.20017
E-2-23	4.9	0.13506	1.75004	1.31920	0.03169	0.15013	0.03063	0.02378	0.00424	0.00306	0.00042	0.26255
E-2-25	5.1	0.17020	2.11568	1.97913	0.06918	0.36721	0.02257	0.01873	0.00485	0.00183	0.00084	0.93584
E-2-26	5	0.16778	12.25062	1.98922	0.04316	0.71962	0.02436	0.02143	0.00553	0.00326	0.00129	0.70224
E-2-28	3.9	0.12851	1.76270	0.75368	0.03629	0.14509	-0.00114	0.00779	0.00093	-0.00018	0.00078	0.23064
E-2-29	6	0.17053	2.43800	2.73217	0.11294	0.35599	0.00139	0.02098	0.00562	0.00238	0.00232	0.39500
E-2-31	5.6	0.17043	2.02240	1.38159	0.06468	0.57042	0.07074	0.05817	0.01798	0.01551	0.00108	0.34523
E-2-32	5.5	0.15020	1.89426	1.37457	0.04944	0.46573	0.05455	0.03836	0.00604	0.00509	0.00131	0.64030
E-2-34	5.9	0.15550	2.04965	1.34170	0.05304	0.17498	-0.00228	0.01106	0.00158	0.00116	0.00079	0.28241
E-2-36	4.3	0.13453	1.70331	2.40144	0.04466	0.23260	0.21593	0.01644	0.00116	0.00199	0.00110	0.30352
E-2-37	6.6	0.15000	1.83213	2.81486	0.07867	0.42147	0.00609	0.01224	0.00259	0.00036	0.00181	0.43561
E-2-38	3	0.13157	1.92339	1.73271	0.03324	0.18158	0.60798	0.03669	0.00489	0.01432	0.00066	181.71422
E-2-39	5	0.15506	1.63113	1.91585	0.07092	0.21928	0.01095	0.00792	0.00252	0.00261	0.00140	0.63774

Table 8 - Entrainment Data Set#3 2001 (ng element per individual larvae)

Sample	L(mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
E-3-1	5.3	0.15468	2.01712	1.36251	0.05107	0.71041	-0.00473	0.00669	0.00126	0.00004	0.01137	0.81783
E-3-2	4.8	0.13395	1.78515	1.39815	0.04423	0.24898	-0.00701	0.00676	0.00078	-0.00052	0.00191	0.65272
E-3-3	5.7	0.14149	1.74418	2.42108	0.05573	0.09947	-0.01044	0.00435	0.00001	-0.00106	0.00170	0.28756
E-3-6	7	0.18097	2.05871	4.03474	0.07943	0.46052	0.08117	0.04690	0.00978	0.00935	0.00264	0.93663
E-3-8	6.2	0.18238	1.93193	1.67647	0.05361	0.14141	-0.00775	0.00617	0.00118	-0.00140	0.00128	0.65075
E-3-11	6	0.16021	2.03924	1.65727	0.06612	1.00641	0.03601	0.03372	0.00529	0.00529	0.00368	0.47976
E-3-13	3.5	0.15774	2.10124	1.16524	0.04306	0.14956	0.00049	0.00492	0.00145	0.00004	0.00081	0.35220
E-3-16	5.2	0.16989	2.18728	2.16280	0.05538	0.41324	0.02651	0.03098	0.00412	0.00151	-0.00072	1.25820
E-3-17	6.1	0.17982	2.36576	1.77837	0.06153	0.29878	-0.05118	0.04057	0.00758	0.00693	0.00456	1.15482
E-3-20	6.4	0.15843	2.06476	1.60024	0.05988	0.23924	-0.00348	0.00889	0.00174	-0.00082	0.00102	0.38382
E-3-21	6.8	0.21113	2.19338	3.28517	0.08003	0.72394	0.02456	0.03048	0.00479	0.00169	0.00241	0.60710
E-3-22	6.2	0.12674	1.74148	2.53244	0.07597	0.19855	-0.00850	0.00594	0.00070	-0.00132	0.00088	0.48214
E-3-23	6.6	0.16805	2.03355	1.97330	0.08074	0.35793	0.01648	0.02105	0.00319	0.00257	0.00165	0.53523
E-3-24	5.7	0.15043	2.01556	1.18079	0.05945	0.23069	0.03921	0.02815	0.00557	0.00498	0.00056	0.44226
E-3-26	6.1	0.13046	1.85588	1.19821	0.05939	0.15041	-0.00845	0.00714	0.00097	-0.00019	0.00157	0.32899
E-3-28	6.6	0.13834	1.95084	1.50896	0.06002	0.29887	0.01963	0.01235	0.00225	0.00176	0.00074	0.85193
E-3-30	6.8	0.28012	2.39269	4.36566	0.09450	3.62425	0.11170	0.07367	0.01439	0.01104	0.00180	2.27897
E-3-31	5.7	0.16837	2.87955	1.65542	0.06919	0.20590	-0.00720	0.00911	0.00214	-0.00090	0.00195	0.25962
E-3-32	6.3	0.14603	2.15485	1.40871	0.06088	0.20358	0.02751	0.02367	0.00472	0.00294	0.00131	0.44089
E-3-33	7.2	0.20327	2.37058	1.96625	0.06116	0.40513	0.10928	0.06572	0.01111	0.00938	0.00096	0.46491
E-3-34	6.3	0.27719	2.46869	1.63403	0.07057	0.28170	-0.00130	0.01230	0.00172	0.00004	0.00146	0.69810
E-3-35	5.7	0.21012	2.25783	1.97107	0.07547	0.51200	0.02353	0.02972	0.00596	0.00535	0.00113	0.56556
E-3-36	4.8	0.11677	1.60378	1.12549	0.04371	0.05871	-0.01357	0.00293	0.00060	-0.00177	0.00101	0.25295

Table 9 - Entrainment Data Set#4 2001 (ng element per individual larvae)

Sample	L(mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
E-4-1	7.7	0.28437	1.86187	1.62875	0.10233	0.57385	0.04712	0.03330	0.00783	0.00665	0.00088	0.42485
E-4-2	8.2	0.24109	1.88275	1.59764	0.08390	0.32206	0.00859	0.01397	0.00371	0.00170	0.00169	0.22398
E-4-3	8.5	0.14324	1.61789	1.67873	0.07473	0.59013	0.12197	0.07430	0.01304	0.01404	0.00128	0.25116
E-4-5	6.2	0.14938	1.68891	46.94319	0.06930	0.82521	0.03263	0.03317	0.00626	0.00409	0.00073	0.48745
E-4-6	6.5	3.49811	3.77235	1.52351	0.06760	0.64156	0.01493	0.01702	0.00422	0.00341	0.00140	0.30839
E-4-7	6.1	0.13335	1.90361	1.51262	0.05695	0.41039	0.04892	0.04416	0.00829	0.00606	0.00147	0.50138
E-4-8	6.8	0.11685	1.52143	3.34680	0.05275	0.28044	0.01406	0.01979	0.00379	0.00177	0.00103	0.26290
E-4-9	6	0.14683	1.82079	1.52332	0.05216	0.58977	0.02064	0.02238	0.00466	0.00179	0.00122	0.30874
E-4-11	6.2	0.14536	1.54225	1.05411	0.04692	0.77377	0.06610	0.04970	0.00950	0.00793	0.00054	0.28807
E-4-12	9.5	0.14740	1.76253	2.33106	0.10184	0.36238	0.00539	0.01659	0.00262	0.00136	0.00176	0.42377
E-4-13	6.2	0.15035	1.58502	1.58555	0.05847	1.40191	0.08716	0.05997	0.01243	0.01157	0.00200	0.48301
E-4-14	8.8	0.15875	1.59667	2.04410	0.06987	0.80712	0.06368	0.05302	0.01191	0.00884	0.00081	0.33899
E-4-15	6.5	0.17424	1.83807	3.40693	0.06975	0.38524	-0.00468	0.00624	0.00153	0.00015	0.00103	0.35909
E-4-16	6.7	0.13554	1.68262	2.15168	0.07726	0.77506	0.03852	0.04150	0.00756	0.00580	0.00130	0.50832
E-4-17	9	0.15439	2.13520	2.76837	0.09723	0.95818	0.11026	0.09899	0.02367	0.02906	0.00247	0.53395
E-4-18	8.1	0.31301	1.86703	3.55295	0.05920	0.70221	0.16443	0.11222	0.02377	0.01890	0.00177	0.34247
E-4-19	8.8	0.14624	2.00692	1.83147	0.15480	0.44704	0.00724	0.01394	0.00207	0.00033	0.00300	0.29308
E-4-20	7.4	0.31621	2.48819	1.79147	0.20362	0.91153	0.01406	0.01756	0.00271	0.00095	0.00107	0.46270
E-4-23	7.9	0.17457	1.82303	1.75424	0.07451	0.50408	0.02162	0.02058	0.00360	0.00086	0.00076	0.37808
E-4-24	4.9	0.13314	1.84537	0.80391	0.04238	0.15410	-0.00190	0.00992	0.00164	0.00035	0.00043	0.20574
E-4-25	6.7	0.13010	1.82165	0.95591	0.04167	0.25405	0.03234	0.02587	0.00563	0.00444	0.00046	0.50583
E-4-26	6.8	0.14819	2.41106	4.37959	0.10709	0.54043	0.05183	0.04062	0.00642	0.00440	0.00075	0.67774
E-4-28	6.4	0.12249	1.64156	1.41786	0.05385	1.13493	0.08781	0.02544	0.00387	0.00466	0.00031	0.36448
E-4-30	6.4	0.12903	1.67768	1.53805	0.06425	0.44983	0.00079	0.01406	0.00309	0.00084	0.00179	0.28018

Table 10 - Entrainment Data Set#5 2001 (ng element per individual larvae)

Sample	L(mm)	Co	Ni	Cu	Mo	Ba	Ce	Nd	Sm	Gd	Au	Pb
E-5-1	7.3	0.1354	1.5668	2.0217	0.0773	0.3635	0.0089	0.0160	0.0029	0.0005	0.0016	0.6591
E-5-2	7.4	0.1669	1.8641	5.0387	0.1027	0.9504	0.0649	0.0480	0.0110	0.0078	0.0023	1.0204
E-5-3	5.5	0.1106	1.4430	0.9640	0.0441	0.1962	-0.0063	0.0034	0.0012	-0.0002	0.0008	0.3584
E-5-4	7.5	0.1457	1.5339	1.6873	0.0828	0.3068	0.0601	0.0442	0.0033	0.0047	0.0007	0.5709
E-5-5	8.5	0.1395	1.5387	2.3544	0.0863	0.2498	0.0055	0.0116	0.0026	0.0003	0.0011	0.4583
E-5-6	8	0.1428	1.5434	1.9686	0.0940	0.2306	-0.0101	0.0045	0.0004	-0.0012	0.0010	0.4056
E-5-7		0.1209	1.5820	0.7705	0.0454	0.1556	0.0277	0.0218	0.0044	0.0025	0.0004	0.2843
E-5-8	7.5	0.1790	1.5129	2.0325	0.1095	0.2699	-0.0020	0.0109	0.0013	-0.0007	0.0009	0.5223
E-5-9	6.6	0.1181	1.4381	2.6291	0.0751	0.2543	0.0040	0.0171	0.0021	0.0010	0.0010	0.5010
E-5-11		0.1108	1.4120	1.2087	0.0628	0.1381	-0.0082	0.0044	0.0001	-0.0012	0.0004	0.2824
E-5-12	7.7	0.1362	1.7489	2.0714	0.1029	0.4263	0.0387	0.0320	0.0056	0.0064	0.0010	0.5625
E-5-13	7.8	0.1210	1.6197	1.8682	0.0901	0.2440	0.0002	0.0155	0.0016	0.0010	0.0020	0.6892
E-5-14	5.1	0.1492	1.8915	1.7584	0.0557	0.2438	0.0092	0.0128	0.0028	0.0001	0.0006	1.0619
E-5-15	5.3	0.1884	1.7268	1.2027	0.0526	0.0934	-0.0115	0.0020	0.0012	-0.0016	0.0003	0.2400
E-5-16	9	0.1670	1.6166	2.7409	0.1114	0.3299	0.0052	0.0086	0.0017	-0.0012	0.0018	0.5691
E-5-18		0.1200	1.7982	0.6998	0.0454	0.0570	-0.0097	0.0029	0.0002	-0.0014	0.0004	0.2251
E-5-19	8	0.1769	1.6903	1.9549	0.0996	0.3178	0.0023	0.0160	0.0019	0.0005	0.0009	0.8143
E-5-20	5.5	0.2883	1.7893	1.0769	0.0939	0.4012	0.0041	0.0171	0.0032	0.0002	0.0010	0.3274
E-5-21		0.1200	1.5684	1.0254	0.0603	0.0790	-0.0052	0.0047	0.0001	-0.0017	0.0006	0.2902
E-5-22	3.8	0.1629	2.0134	2.3027	0.0593	0.2304	0.0588	0.0277	0.0076	0.0070	0.0013	1.0308
E-5-24	7.8	0.1473	2.2785	1.6516	0.0618	0.2798	0.0061	0.0152	0.0016	0.0019	0.0012	1.4240

Table 11 - 2001 nonparametic discriminant function with K=2, training data

Source	NR	TR	WB	Other	Total
NR	32	4	2	4	42
	76.2%	9.5%	4.8%	9.5%	100%
TR	0	23	6	3	32
	0.0%	71.9%	18.8%	9.4%	100%
WB	2	3	35	3	43
	4.7%	7.0%	81.4%	7.0%	100%
Total	34	30	43	10	117
	29.1%	25.6%	36.8%	8.5%	100%

Table 12 - 2001 NeuroShell Easy Classifier™ training data

Source	NR-actual	TR-actual	WB-actual	Total
NR-clasified as	39	2	6	47
TR-clasified as	2	27	1	30
WB-clasified as	1	3	36	40
Total	42	32	43	117
Sensitivity	92.9%	84.4%	83.7%	

Table 13 - 2001 micro-elemental and DNA entrainment data analysis results summary

Source	NR	TR	WB	Other	Total
Nonparametric discriminant function with K=2, entrainment data	5 4.4%	0 0.0%	74 65.5%	34 30.1%	113 100%
Nonparametric discriminant function with kernal density estimates with equal bandwidth	16 14.2%	6 5.3%	91 80.5%	0 0.0%	113 100%
NeuroShell Easy Classifier™ entrainment data, cut-off = 0.65	12 10.6%	2 1.8%	37 32.7%	62 54.9%	113 100%
NeuroShell Easy Classifier™ entrainment data, cut-off = 0.5	23 20.4%	4 3.5%	69 61.1%	17 15.0%	113 100%
Nuclear DNA NeuroShell Easy Classifier™	260 24.4%	236 22.1%	368 34.5%	203 19.0%	1067 100%