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Ecology and dynamics of river herring larvae in the Pamunkey River. Virginia: : June 1, 1989 - December 31, 1991

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ERRATA

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22	Chi-square values indicated as 'X' should read 'X^2'.
22	Change 'pronounce' to 'pronounced'.
41	Table 1-11. Change 'prolarvae' to 'postlarvae', change 'prolarva' to 'postlarva'.
134	Change '(Sismour and Loesch, in review)' to '(Appendix A)'.
146	Change 'by Sismour and Loesch (in review)' to 'in Appendix A'.
172	Change 'Sismour' to 'Sismour[1]'.
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ABSTRACT

The early life histories of anadromous herrings (*Alosa* species) in tidal freshwater are poorly understood. Knowledge of the ecology of anadromous herring larvae in tidal freshwater ecosystems is important in order to understand factors which cause population fluctuations, to mitigate potential adverse effects of modifications to these systems, and to facilitate restoration of populations. This investigation was undertaken to examine the distribution and relative abundance of larval river herring (alewife, *Alosa pseudoharengus*, and blueback herring, *A. aestivalis*) and their potential zooplankton prey in tidal freshwater of the Pamunkey River, Virginia, and to quantify growth of larvae between locations that may differ in prey availability within the study area.

Herring larvae were distributed throughout the study area along the river main-stem. Herring preflexion postlarvae were distributed in all potential habitats examined. Postflexion larvae, however, were most common in Holts Creek, a tidal creek located in the lower reach of the study area. Larger larvae were more common in collections made in Holts Creek than at other sites, and they were consistently captured from this creek during early May. The occurrence of a high number of postflexion larvae during a relatively short time interval suggests that Holts Creek, and possibly other tidal creeks, may be utilized as nursery grounds for a relative brief duration during early life. Relative densities of potential zooplankton prey increased with distance downstream, and were higher in two tidal creeks sampled compared to the river main-stem or to Cumberland Thoroughfare, a secondary channel.

An effort was made to rear alewife and blueback herring larvae from eggs. Larvae reared from eggs were used to validate the rate of otolith increment deposition and to evaluate meristic, morphometric, and pigmentation characters for use as taxonomic criteria to delimit these species in field collections. From this work, a method was developed for culturing river herring larvae from eggs. The taxonomic study demonstrates that meristic and morphometric methods are insufficient to delimit larvae, but pigmentation differences delimit postflexion larvae. The age validation study supports the hypothesis of daily increment deposition in these species, and provides the basis for applying the otolith increment method to field collected specimens. Due to time limitation, however, studies of the dynamics of larval river herring in the Pamunkey River were not accomplished.

PREFACE

This report is subdivided into three parts and three appendices. Parts I and II present results of a field study examining the distribution and abundance of the early life stages of herring and the potential prey of herring postlarvae in the Pamunkey River, Virginia. Summaries of these sections are located on pages 4-5 and 67, respectively. Part III presents the results of a study to validate increment deposition in otoliths of cultured river herring larvae. The summary for this section is located on page 97.

The three appendices of this report are draft manuscripts. Appendix A details a continuous-flow culture system used to rear river herring larvae, and Appendix B documents the results obtained using this system. Appendix C presents a study of morphometry, meristics, and pigmentation of cultured alewife and blueback herring larvae to identify methods for delimiting these species in field collections.

This report forms the basis of doctoral dissertation research conducted by the primary author.

ACKNOWLEDGMENTS

The contributions of all who participated in this study are gratefully acknowledged. This work could not have been completed without the assistance provided by staff and students of the Virginia Institute of Marine Science (VIMS) and by friends and family of the primary author. Donna Paterson, Holly Marshall, and Terry Holland assisted in sorting ichthyoplankton collections, and Donna Paterson processed the zooplankton samples. The authors thank Dr. Cynthia Jones, Old Dominion University, Norfolk, Virginia, for technical guidance and assistance in the analysis of larval herring otoliths, and Mr. John Olney, VIMS, for access to laboratory and field equipment used for various components of this study.

Special recognition is given to Bob and Rose Windsor who permitted use of their property for the culture of river herring larvae in 1990 and 1991, and for their hospitality and generosity.

PROJECT OVERVIEW

The early life histories of anadromous herrings (Alosa species) in tidal freshwater zones of tributaries to the Chesapeake Bay are poorly understood. In comparison to studies of the adult and juvenile stages, relatively little effort has been made investigating the ecology of the larval stage of these species. This gap is important to address both from a fisheries perspective and from an ecological perspective.

Fundamental objectives of fisheries research included understanding why fluctuations in populations of a species occur and how the recruitment process differs between species. Females of most fishes produce a superabundance of eggs and relatively few survive to complete the life cycle. High mortality occurs during the egg and larval stages so that the size of a year class is established by the juvenile stage. How and why individuals survive is an important question, and it is likely to be associated with the distribution of eggs and larvae in their environment.

Tidal freshwater ecosystems utilized by the anadromous herrings for nursery habitat are poorly understood. The functional significance of herring in tidal freshwater ecosystems is important to understand since relatively few species utilize this region for nursery habitat to the same extent as herring. An understanding of the ecology of anadromous herring in tidal freshwater ecosystems is important in order to understand factors which cause population fluctuations, to mitigate potential adverse effects of modifications to these systems, and to facilitate restoration of populations.

This investigation was undertaken to investigate the distribution and relative abundance of river herring (alewife, *Alosa pseudoharengus*, and blueback herring, *A. aestivalis*) and their potential zooplankton prey in tidal freshwater of the Pamunkey River, Virginia, and to quantify growth of larvae between locations that may differ in prey availability within tidal freshwater of the Pamunkey River, Virginia.

The investigation was conducted to evaluate the distribution and relative abundance, and age and growth of these species within a predetermined reach of the river main-stem from the upper estuary into tidal freshwater and between areas hypothesized to represent distinct habitats.

A goal of the investigation was to compare the distributions of alewife and blueback herring larvae; however, this was not successful. A study was conducted using alewife and blueback herring larvae reared from eggs of known taxonomy (Appendices A and B) to determine whether meristic and morphometric characters delimit these species. The results suggest that meristic and morphometric characters are of little or no value for identifying these taxa, but pigmentation differences were found that can delimit these species once flexion has occurred (see Appendix C).

Another complication to the study of the ecology of river herring larvae in tidal freshwater systems in the Chesapeake Bay is the coincident distribution of gizzard shad (*Dorosoma cepedianum*) eggs and larvae. River herring and gizzard shad prolarvae are readily identified based on morphology of the yolk sac; however, postlarvae of these groups are nearly identical until fin formation is complete. Prior to complete fin formation, postlarvae of these taxa differ in the ratio of the vent length to the standard length, although some overlap may exist. Pigmentation characteristics may exist that might delimit gizzard shad from river herring, but this has not been investigated. Consequently, this report examines the distribution and relative density of herring eggs, river herring and gizzard shad prolarvae, and herring postlarvae; when possible, distributions of preflexion and postflexion larvae are compared (Part I).

River herring larvae are known to ingest rotifers, copepod nauplii and cladocerans, although the relative significance of each potential prey group may differ between alewife and blueback herring. Due to time

limitations, feeding habits of herring larvae were not examined for this report; however, guts of wild herring postlarvae and cultured alewife and blueback herring were observed to contain cladocera and the rotifer Keratella spp. Distributions of rotifer, cladoceran and copepod potential prey were examined in the study area along the river main-stem and between potential habitats (Part II).

An important aspect of the distribution and abundance of herring larvae is whether growth and survival vary between areas which differ in the availability of potential prey. Daily deposition of growth increments in otoliths provides a potential method for evaluating the age and growth of herring larvae captured at different locations within the tidal freshwater system. Before applying the daily increment method to field-collected larvae, however, a necessary prerequisite is validation of the rate of increment formation. For the present report, increments in otoliths from river herring larvae cultured in 1990 and from culture alewife and blueback herring in 1991 were enumerated and analyzed to determine whether increments were deposited daily (Part III).

The present investigation provides a preliminary assessment of the ecology and early life history or river herring in a tidal freshwater ecosystem. Potential benefits include:

1. increased knowledge of the distribution of river herring larvae and their potential zooplankton prey in a tidal freshwater system and the relative significance of various habitat types to the early life stages of river herring;
2. a basic design of a system for the culture of alewife and blueback herring postlarvae to facilitate experimental investigations of the early life stages;
3. a method for the identification of alewife and blueback herring postflexion larvae; and,
4. validation of increment deposition in otoliths of known-age alewife and blueback herring larvae.

PART I. Distributions of Herring Larvae in Tidal Freshwater of the Pamunkey River, Virginia.

SUMMARY

1. Herring early life stages were sampled from the tidal freshwater reach of the Pamunkey River in 1989 and 1990 in the zone from mile (nautical) P45 to P62, inclusive (measured from the mouth of the York River), and from five potential habitats in and adjacent to the zone from P53 to P54. The potential habitats included: the river channel, the river shoulder (flats) adjacent to the channel, Cumberland Thoroughfare, and two tidal creeks, Holts Creek and Big Creek. Oblique tows were conducted to sample the water column of the river main-stem and Cumberland Thoroughfare, and a pushnet sampled the near-surface water of potential habitats.

2. During the 1989 season, sixteen sampling events were conducted from mid-March to the end of May to collect ichthyoplankton from the river main-stem. Additional samples were collected in Cumberland Thoroughfare. Three sampling events were conducted from mid-April to the end of May to collect ichthyoplankton from potential habitats.

3. During the 1990 season, greater effort was expended sampling in the potential habitats to more completely characterize the utilization of the tidal creeks by herring larvae. Seven sampling events were conducted from mid-March to mid-May to collect ichthyoplankton from potential habitats, and four sampling events were conducted from mid-March to mid-May to collect ichthyoplankton from the river main-stem and Cumberland Thoroughfare.

4. The 1990 season was relatively warmer than the 1989 season. Temperature differences between the two seasons were greater in March than in April or May. Herring eggs, river herring prolarvae, gizzard shad prolarvae, and herring postlarvae were captured several weeks earlier in 1990 compared to 1989.

5. Relative densities of herring eggs were often higher in Cumberland Thoroughfare compared to the river main-stem. High relative densities of herring eggs in the river main-stem were observed in the zones from P45 to P47 and from P57 to P62 during both sampling seasons.

6. Few herring eggs were captured in near-surface waters in the potential habitats. River herring and gizzard shad eggs are semi-adhesive and range from semi-demersal to pelagic, and it is likely that few eggs were entrained in near-surface water.

7. River herring prolarvae were captured from early April to late May in 1989 and from mid-March to May in 1990. Mean relative densities were highest in late April during both sampling seasons. Relative densities were more often higher in Cumberland Thoroughfare than in the river main-stem. Mean relative densities appeared to be lower in 1990 compared to 1989.

8. Relative densities of prolarvae appeared to be higher in tidal creeks in 1990. Greater relative density in the vicinity of Cumberland Thoroughfare in 1990 may indicate greater spawning activity in the lower tidal freshwater reach. Warmer water

temperature in 1990 may have induced adults to mature earlier and seek spawning sites lower in the tidal freshwater reach.

9. Gizzard shad prolarvae were captured primarily after mid-April in both seasons. Relative densities were often higher in Cumberland Thoroughfare compared to the river main-stem. Mean relative densities in the river main-stem were comparable during similar time periods of the two seasons. Peak relative densities occurred in the zone from P48 to P59 in 1989, and were highest in the zone from P57 to P62 in 1990.

10. Gizzard shad prolarvae were initially captured only in tidal creeks; but, as the season progressed, they were captured in all potential habitats. Highest relative densities were typically observed in the tidal creeks. Higher relative densities in tidal creeks may indicate greater use of these areas for spawning by gizzard shad compared to river herring.

11. Herring postlarvae captured in the river main-stem were primarily preflexion larvae. Peak relative densities were observed in late April-early May during both seasons, and were observed in the zone from P48 to P59 in 1989 and from P57 to P59 in 1990. Relative densities were more often higher in the river main-stem compared to Cumberland Thoroughfare.

12. Preflexion larvae were captured in all potential habitats. In comparison, postflexion larvae (including larvae undergoing flexion) were captured primarily in Holts Creek.

13. The percent distribution of herring postlarvae in potential habitats during mid- to late April differed between the two seasons. The difference is suggested to be associated with earlier movement of larvae into tidal creeks in 1989. By May, however, the percentage distribution of postlarvae between potential habitats was markedly similar during both seasons, despite differences in relative densities observed between sampling events both within and between years.

14. Herring postflexion larvae appeared to vary in their diel distribution within Holts Creek. Relative densities of larvae were higher earlier in the day compared to later in the day.

15. Identification of alewife, blueback herring, and gizzard shad postlarvae was not possible because of conflicting information in the literature. A study was conducted to examine characteristics identified in the literature as diagnostic for alewife and blueback herring. A continuous-flow culture system was designed and, in 1991, alewife and blueback herring larvae were reared from eggs of known taxonomic identity. Results indicate that morphometric and meristic characteristics are of little value for the identification of alewife and blueback herring postlarvae, but pigmentation characters differ between postflexion larvae of these species.

PART I. Distributions of Herring Larvae in Tidal Freshwater of the Pamunkey River, Virginia.

Objective: Characterize the temporal and spatial distributions of river herring larvae in tidal freshwater of the Pamunkey River, Virginia.

INTRODUCTION

Persistence of a biological population through time is a function of the capacity of the parental stock to produce new individuals and of the survival of these individuals until they recruit to the mature population. Its abundance is controlled through differential rates of recruitment between year classes as a function of the interactions between it and the biotic and abiotic components of the ecosystem. A detailed understanding of the processes which regulate recruitment is lacking for most, if not all, fish populations.

Numerous hypotheses have been advanced to account for recruitment variability in fish populations (reviewed by May 1974, Anderson 1988). Studies of the early life history of American shad (*Alosa sapidissima*) in the Connecticut River and of its population dynamics provide a conceptual basis for the examination of recruitment of *Alosa* in other systems (Leggett 1976, 1977; Crecco and Savoy 1987a, 1987b). The recruitment process for populations of *Alosa* from various regions may be similar in many respects; however, differences between populations may occur as a consequence of population-specific life history adaptations. Species-specific and population-specific variations in adult biology (e.g. sex ratio, age structure), life-history strategies (e.g. semelparity or iteroparity), and behavior (e.g. selection of spawning habitat, timing of spawning events), as well as differences in the biology of larvae and juveniles (e.g. behavior, prey utilization, habitat utilization) necessitate investigation of *Alosa* populations in different systems to enable the development of a comprehensive understanding of the factors affecting recruitment and abundance for populations within this group.

A greater understanding of tidal freshwater systems as nursery habitat for fishes will be essential to provide appropriate balance between increased utilization by an expanding human population and habitat requirements for species utilizing tidal freshwater habitats during some or all of their life-history. In the Virginia portion of the Chesapeake Bay watershed, the human population is expected to increase from 4.7 million to over 6.2 million by the year 2020 (Anon. 1989a). A substantial portion of this growth is projected to occur along the western shore of the bay in the corridor from Northern Virginia to Hampton Roads where Virginia's tidal freshwater systems are found, and municipalities increasingly view tidal freshwater as a water source to support the growth.

In the Chesapeake Bay watershed, alewife and blueback herring (collectively referred to as river herring) and other anadromous species utilize tidal freshwater for spawning and nursery habitat (Odum et al. 1984). The early life history of river herring in tidal freshwater systems and of the biotic and abiotic factors that influence growth and survival are poorly understood. River herring larvae are widely distributed in the upstream reach of the York-Pamunkey estuary, but the relative value of the upper estuary compared to tidal freshwater as a nursery zone or on the utilization of out-of-channel areas by herring larvae is not well known.

The present study was undertaken to characterize the distribution of herring larvae in the Pamunkey River, Virginia, in tidal freshwater from the upper York-Pamunkey estuary as defined by Van Engel and Joseph (1968) to tidal freshwater upstream of estuarine influence, and between areas hypothesized to represent distinct habitats.

METHODS

Study area.

The Pamunkey River, located in east-central Virginia (Fig. 1-1), flows approximately 85 nautical miles following the main-stem in a southeasterly direction from its origin at the confluence of the North Anna and South Anna rivers, near Hanover, Virginia, to its confluence with the Mattaponi River at West Point, Virginia (Brooks 1983). The confluence of the Mattaponi and Pamunkey rivers occurs approximately 29 nautical miles (P29) upstream from the mouth of the York River in Chesapeake Bay. The Pamunkey River is under tidal influence for most of its length. Van Engel and Joseph (1968) defined the upper limit of the York-Pamunkey estuary at approximately P50. Van Engel and Joseph (1968) reported mean salinity of 0.2‰. (0.0 to 1.8‰.) at this level with lowest salinities occurring in winter and spring. Lower salinities at this time are associated with higher runoff, although tidal conditions also influence the position of the salt-fresh interface. Head-of tide, the maximum upstream extent of tidal influence, is located about P74. The river drains approximately 3,781 square kilometers (2,349 square miles) of primarily agricultural lands and extensive marshes, and little industrial development occurs above the West Point area (Brooks 1983). Along most of its length, the river is bordered by marshes and forested wetland.

The Pamunkey River is a typical flood-plain river following a sinuous route from narrow and relatively shallower conditions upstream to wide and relatively deeper conditions downstream. The lower river channel is characterized by extensive meandering. Secondary river channels that transect marshes on the inner side of several meanders are referred to as thoroughfares. Cumberland Thoroughfare is the largest thoroughfare in the Pamunkey River and joins P49 to P54.

The tidal-freshwater zone of the Pamunkey River between P45 and P62, inclusive, was selected as the study area (Fig. 1-2).

Ichthyoplankton and zooplankton collections (see Part II) were made along the river main-stem and in Cumberland Thoroughfare to characterize the longitudinal distribution of herring larvae and their potential prey in this zone. Ichthyoplankton and zooplankton collections were also made in areas adjacent to the river main-stem channel to establish whether clupeid larvae utilized these areas. Potential habitats selected for this aspect of the study were located in the vicinity of Cumberland Thoroughfare (Fig. 1-3a) and included: the river channel, shoal areas adjacent to the channel, Cumberland Thoroughfare, and two tidal creeks, Holts Creek and Big Creek.

Holts Creek and Big Creek drain Lilly Point marsh (Fig. 1-3b) which is characterized by extensive emergent vegetation consisting primarily of arrow-arum (*Peltandra virginica*) and yellow pond lilly (*Nuphar luteum*). The marsh is bordered on the southeastern side by Holts Creek, and Big Creek occurs in the center. Holts Creek is restricted by a ridge lined with forest and shrub and grain cultivation occurs beyond the tree line. Because it flows against this ridge, Holts Creek is relatively straight with a single meander. Big Creek bisects Lilly Point Marsh and demonstrates a greater degree of meandering. Holts Creek and Big Creek flow in the same general direction, are separated by a distance ranging from one-fifth to one-half mile, and neither exhibits extensive subdivision into smaller tributaries. Topographic maps indicate possible communication between the two tidal creeks by small rills which may be permanent or inundated only during high tide; the extent of exchange between the two creeks is not known. Holts Creek empties into Cumberland Thoroughfare and Big Creek empties into the Pamunkey River main-stem.

Study design.

Ichthyoplankton and zooplankton were collected during the spawning seasons of river herring in 1989 and 1990. Intensive sampling during

both seasons using two different methods to collect ichthyoplankton along the river main-stem and in potential habitats was not possible; consequently, greater sampling effort was made along the river main-stem during 1989 and greater sampling effort was made between potential habitats during 1990.

Sampling among strata along the river main-stem in 1989 was conducted weekly from mid- to late March; preliminary sampling in 1988 suggested that little spawning occurs during this period of the season. Samples were collected twice-weekly from early April to early May during the period when greatest spawning activity was expected, and sampling during mid- to late May was conducted at intervals of about 5 days. Sampling in potential habitats during 1989 was conducted on 2 April, 20 April, 18 May, and 30 May. The 2 April collection was incomplete (a single collection from Holts Creek) and it was not included for analysis. In 1990, sampling along the river main-stem was reduced to half-monthly collections from mid-March to mid-May, and potential habitats were sampled once in mid-March and weekly from April to mid-May.

A stratified-random sampling design was used to determine the locations for sampling. The river main-stem was divided into six consecutive blocks consisting of three one-mile substrata to ensure that collections were obtained throughout the area for each sampling event. One substratum from each block was selected randomly for each event, and a single collection was taken from each block. Additional collections were made in Cumberland Thoroughfare to determine whether this area might be of importance to early life stages of herring. Collections in Cumberland Thoroughfare using the bongo nets were not planned as part of the original study and must be considered auxiliary, since the thoroughfare could not be subdivided into strata equivalent to those along the river main-stem. Collections from the thoroughfare were obtained weekly in early April 1989, but were obtained during each

sampling event from 18 April to 25 May in 1989 and during all sampling events in 1990. Samples were typically collected from near the center of the selected substrata and near the center of the thoroughfare.

Ichthyoplankton along the river main-stem were collected using 333μ-mesh plankton nets (5:1) towed from a 21 ft Privateer inboard-outboard with a 155 hp commercial Evinrude outboard engine and fitted with a boom and winch (Fig. 1-4). The plankton nets were attached to a bongo-net frame consisting of two 60 cm diameter (0.36 m) rings and weighted with about 40 lb. Flow meters were positioned in the mouth of each net, approximately half the distance between the center and rim (Tranter and Smith, 1968) to measure water volume filtered. The water column was sampled using stepped-oblique tows from the surface proceeding at two-meter depth intervals to within one to two meters of the substrate. Each depth interval, including the surface, was sampled for 30 seconds, and the assembly was retrieved from the lowest depth interval without stopping. Boat speed during tows was maintained at 750 to 800 RPM, the slowest engine speed possible while making way. Water depth was monitored electronically using a depth finder with a liquid-crystal display (LCD). A tow was repeated if it were possible that the nets had dredged the bottom or hit a snag.

A different method was necessary to sample ichthyoplankton in the tidal creeks because the Privateer could not easily maneuver in Holts Creek and it could not enter into Big Creek due to shoaling at the mouth. Collections in potential habitats were made using a 14 ft jon boat, powered with 25 hp Johnson outboard motor, to which a pushnet frame was attached (Fig. 1-5). The pushnet frame consisted of a rectangular guide constructed from 31.8 mm-channel aluminum in which fit a polyvinyl chloride (PVC) frame to which plankton nets were lashed. Side supports for the frame were constructed from 50.8 mm diameter aluminum tube. Near the front and back of the frame, the side pieces were braced with aluminum tube that spanned the width of the jon boat.

The frame was bolted to the top of the gunnel about 1.5 m behind the bow. The side supports were bent so that the plankton nets were perpendicular to the water surface and its bottom was 1 m below the surface during towing. The frame was held in position during towing and was raised and lowered using ropes. Two 60 cm 335μ-mesh plankton nets (5:1) were lashed to a net-support frame constructed from 25.4 mm diameter PVC pipe with two 42 cm by 35 cm (0.15 m^2) openings. The PVC frame slid into the rectangular aluminum frame and was held in place using duct tape. Flow meters were positioned about one-third the height of the frame from the bottom in the mouth of each net. Each tow was conducted for five minutes at minimum engine speed.

Sampling locations in and adjacent to the river main-stem channel were selected to provide a cross-section of presumed habitat types (Fig. 1-3a). Sampling sites in Holts Creek and Big Creek consisted of a one-nautical mile interval beginning one nautical mile from the mouth of each creek subdivided into two one-half mile strata. Cumberland Thoroughfare was divided into two strata, one upstream and the other downstream of the mouth of Holts Creek. Upstream and downstream strata for sampling locations in the river channel (referred to as Channel) and over the shoals adjacent to the channel (referred to as Flats) were located in P53 and P54. Collections were made over the river shoulder on the eastern side of the channel in P53 and on the northern side of the river channel across from the mouth of Big Creek in P54. Collections made in the river channel were taken in areas adjacent to the river shoulder areas just described. One stratum of each habitat was selected randomly for each sampling event and replicate samples were obtained. One tow was made with the tide and one was made against the tide to average any affect of tide on catch-per-effort estimates. If samples were made during slack water, one tow was made proceeding upstream and the other proceeding downstream. In 1990, collections were

made in Holts Creek relatively earlier and relatively later in the day to determine whether the time of day might influence catch-per-effort.

Paired ichthyoplankton samples were obtained during each tow to provide specimens for other aspects of this project, including specimens for analysis of age and growth and analysis of nutritional condition. One sample of each pair was always fixed in 5% phosphate buffered formalin (5% PBF) (Markle 1984), while the other was fixed either in 95% ethanol (EtOH), to preserve otoliths, or in Bouins solution for histological analysis of nutritional condition. Ethanol-fixed samples were changed twice at intervals of approximately 24 hours to ensure complete fixation of larvae and preservation of otoliths. Samples fixed in Bouins solution were transferred to 70 percent ethanol after 24 hours.

Ichthyoplankton samples were sorted to remove all fish eggs and larvae. Larvae from samples preserved in 5% PBF were identified to lowest possible taxon, and were enumerated. Larvae from the other collections were archived for future use. American shad eggs and larvae were identified, and remaining clupeid early life stages were identified as eggs, river herring or gizzard shad prolarvae, or as preflexion or postflexion (includes both flexion and postflexion) larvae. Relative densities of fish larvae were calculated by adjusting the number of larvae in each sample to a standard volume of 100 m^3. Relative densities of river herring and gizzard shad postlarvae were estimated for each sampling season based on the percentage of prolarvae of each group during each sampling event multiplied by relative density of postlarvae.

Field-measurements included time of day, tide direction, water transparency measured using a secchi disc, and water temperature and dissolved oxygen concentration measured using a portable YSI temperature-dissolved oxygen meter at a standard depth of 1 meter.

Daily air temperature for the east-central region of Virginia was obtained from published U.S. Weather Service records taken at R. E. Byrd International Airport, Richmond, Virginia (Anon. 1989b, c, d; Anon. 1990a, b, c). Mean daily velocity of the Pamunkey River was obtained from published records for the National Stream-quality Accounting Network, Station 01673000 located near Hanover, Virginia (latitude 37 46'03", longitude 77 19'57") (Prugh et al. 1990, 1991). Mean daily water temperature in the lower York River were monitored by the Virginia Institute of Marine Science Division of Physical Oceanography and Environmental Engineering.

RESULTS

Environmental conditions.

Environmental conditions during the two sampling seasons differed. Mean seasonal air temperature measured in Richmond and mean seasonal water temperature of the lower York River measured was higher in 1990 compared to 1989 (Table 1-1). Differences in mean monthly temperatures between the two sampling seasons were greatest in March compared to April or May. Mean water temperature within the study area was higher in 1990 with the greatest difference occurring during March (Table 1-2). During both seasons water temperature was generally uniform between strata with fluctuations typically ranging between 0.5 to 1.0 C. Water temperature observed in potential habitats during the two sampling seasons are presented in Table 1-3.

Mean monthly river flow was lower in March and higher in April 1990 compared to 1989, but were similar in May of both seasons (Table 1-1). From 3 May to 12 May, 1989, daily mean river flow equalled or exceeded 3000 cfs, and peak flow during this interval exceeded 8000 cfs on 9 May. Lower river flow in March 1990 compared to March 1989 may indicate a delay of the spring freshet which could have influenced

inputs of dissolved and particulate nutrients and the timing of the spring plankton bloom.

Mean seasonal concentration of dissolved oxygen within the study area did not differ between the two sampling seasons (Table 1-2). During most sampling events, dissolved oxygen concentrations were lower upstream within the study area, but this trend was not always consistent (Table 1-4). Higher dissolved oxygen concentrations downstream in the study area may have occurred due to increased primary productivity in this zone. Increased dissolved oxygen concentration may result from higher productivity due to nutrient inputs associated with elevated river flow, but excessive flow may reduce dissolved oxygen concentration due to high suspended solids (Fig. 1-6). Dissolved oxygen concentration observed in potential habitats during the two sampling seasons are shown in Table 1-5.

Water transparency along the river main-stem ranged from 20 to 129 cm (Fig. 1-7). Highest transparency in 1989 occurred during April, prior to the period of high water flow in early May. In 1990, highest water transparency was observed in late April. Water transparency observed in potential habitats during the two sampling seasons is presented in Table 1-6.

Distribution and relative abundance of Clupeid early life stages.

Herring eggs.

Relative densities of herring eggs captured within the main-stem channel in 1989 and 1990 are summarized in Fig. 1-8. Relative densities of herring eggs were higher in Cumberland Thoroughfare than in strata along the main-stem channel (Table 1-7). In both years, the relative density of eggs was more often greater in Cumberland Thoroughfare than in strata along the river main-stem, and eggs were captured only in the thoroughfare in mid-March 1990.

Few herring eggs were captured in surface waters of the tidal creeks during both years (Table 1-8), except for 30 May 1989. Eggs captured on 30 May 1989 are likely to have been gizzard shad based on the time of year the eggs were captured, but positive confirmation of species identity was not possible. Eggs generally were more frequent in Big Creek than in Holts Creek; however, relative densities in both locations were low. Eggs were captured in Cumberland Thoroughfare during April of both years and during May 1989. Few, if any, eggs were captured in surface waters of the main-stem channel or over shoal areas during both seasons.

River herring prolarvae.

The distributions of river herring larvae along the river main-stem within the study area during the 1989 and 1990 sampling seasons are shown in Fig. 1-9. River herring prolarvae were captured from 28 March to 25 May; however, relative densities between 100 and 500 prolarvae per 100 m^3 were most common in the study area during April, and were typically below 100 prolarvae per 100 m^3 during May, 1989. Highest relative densities, observed during late April-early May, exceeded 1000 prolarvae per 100 m on two occasions in stratum 6. During mid-April relative densities of prolarvae were conspicuously lower in the zone from mile 53 to mile 55 compared other sample sites either upstream or downstream.

Relative densities of river herring prolarvae in the river main-stem were lower in 1990 than during comparable time intervals in 1989, with the exception of 21 March. Elevated densities in March are suggested to have occurred as a consequence of warmer water temperatures which initiated spawning earlier than in 1989. Relative densities of prolarvae in 1990 collections were typically less than 100 larvae per 100 m^3, with the exception of higher relative densities in stratum 6 on

27 April and 9 May. In contrast to collections from April and May 1989 of which only a few were characterized by an absence of prolarvae, 7 of 18 collections (39 percent) during April and May 1990 did not contain river herring prolarvae. In both seasons, the relative densities of prolarvae were often greater in strata near the upstream limit of the sampling area.

Table 1-9 presents the relative densities of river herring prolarvae captured in the river main-stem and in Cumberland Thoroughfare during the two sampling seasons. Table 1-10 summarizes the relationship between relative densities of river herring prolarvae captured in Cumberland Thoroughfare in comparison to relative densities of prolarvae captured in strata of the river main-stem. Similar relationships were observed in both seasons. Relative densities of prolarvae were more often greater in the thoroughfare than in the river main-stem. Table 1-11 summarizes the relationship between relative densities of river herring prolarvae captured in Cumberland Thoroughfare compared to the river main-stem in April and May, 1989. No apparent trends were observed in the relative densities of river herring prolarvae in the thoroughfare compared to strata 1, 2, 3, and 6 during April, but relative density was more often greater in the thoroughfare compared to strata 4 and 5. In May, relative densities of prolarvae were more often greater from strata 3 to 6 compared to the thoroughfare, no trend was observed between stratum 2 and the thoroughfare, and their relative density was more often lower in stratum 1 than in the thoroughfare. Insufficient samples were obtained in 1990 to compare the distributions of river herring prolarvae in April and May.

Relative densities of river herring prolarvae in potential habitats in 1989 and 1990 are presented in Table 1-12. Relative densities in April appear to have been greater in 1990 compared to 1989. Relative densities of river herring prolarvae were low in May during both sampling seasons. In 1990, prolarvae were captured as early as 17

March, but highest relative density occurred in late April. Relative densities of prolarvae appear to have been higher in the tidal creeks in 1990 compared to 1989. Although this apparent difference may have resulted from lower sampling effort in 1989, an alternative explanation suggests that greater relative densities of prolarvae in tidal creeks in the vicinity of Cumberland Thoroughfare may indicate greater utilization of this region for spawning in 1990. Warmer water temperature as experience in 1990 might induce a greater percentage of adults to mature earlier and to seek spawning sites lower in the tidal freshwater reach.

Gizzard shad prolarvae.

Relative densities of gizzard shad prolarvae are examined to document their occurrence and distribution within the study area in relation to that of river herring prolarvae. In both seasons, most gizzard shad prolarvae were captured primarily beginning about 22 April; however, prolarvae were captured in 1990 as early as 17 March in Cumberland Thoroughfare presumably due to warmer water temperature.

The distributions of gizzard shad prolarvae along the river main-stem during the 1989 and 1990 sampling seasons are shown in Fig. 1-10. Mean relative densities of gizzard shad prolarvae in strata along the river main-stem were comparable during similar time periods of the two seasons; however, the distribution of prolarvae between strata appeared to differ. Peak relative densities of prolarvae during late April-early May, 1989, occurred in the zone from stratum 2 to stratum 5. Relative densities of gizzard shad prolarvae in 1990 were higher in strata 5 and 6.

Table 1-13 summarizes the relative densities of gizzard shad prolarvae captured in strata along the river main-stem and in Cumberland Thoroughfare. Relative densities of gizzard shad prolarvae in Cumberland Thoroughfare in 1989 exceeded relative densities in all strata along the channel, and relative densities in the thoroughfare in

1990 were higher than all strata along the channel except strata 2 and 6 (Table 1-14). In both years, relative densities of prolarvae in Cumberland Thoroughfare were more similar to relative densities in strata 2, 4, and 5 than to relative densities in strata 1, 3, and 6.

Gizzard shad prolarvae were captured only in the tidal creeks prior to 22 April in both years, but were captured throughout the study area on this date and thereafter (Table 1-15). Relative densities were typically less than 200 prolarvae per 100 m^3, but higher relative densities were encountered in a few collections. Highest relative densities observed on 29 April 1990 were in the tidal creeks. Higher relative densities of gizzard shad prolarvae in tidal creeks may indicate greater utilization of these areas for spawning by this group compared to river herring.

Clupeidae postlarvae.

The distributions of preflexion larvae along the river main-stem within the study area during the 1989 and 1990 sampling seasons are shown in Fig. 1-11. Few postlarvae captured using 333μ plankton nets along the river main-stem had advanced developmentally beyond the preflexion stage. Relative densities during most of April and May were below 100 larvae per 100 m^3, but higher relative densities occurred from about 25 April to 5 May. Relative densities exceeding 1000 larvae per 100 m^3 were observed during the 3 May to 5 May interval from stratum 2 to stratum 5. Relative densities of postlarvae throughout the study area declined markedly between 5 May and 11 May. A number of factors may have contributed to this decline: a) gear avoidance associated with increased size of larvae, b) emigration from the channel into near-shore or tidal creek habitats, and c) advection from the study area by elevated river flow (<3000 cfs from 3 to 12 May).

Relative densities of postlarvae in 1990 were generally below 100 larvae per 100 m^3. Higher relative densities were found in samples collected on 27 April from stratum 3 to stratum 6. Peak relative density of postlarvae exceeding 1000 postlarvae per 100 m^3 was found in the 27 April collection from stratum 5. Peak relative densities in both 1989 and 1990 were found in the same general region on the study area, but occurred relatively earlier in 1990 compared to 1989 suggesting that the reproductive season might have begun earlier or progressed faster due to warmer water temperature.

Clupeid postlarvae were captured in Cumberland Thoroughfare throughout both sampling seasons (Table 1-16). In 1989, the relative density of postlarvae was more often greater in the river main-stem in downstream strata within the study area compared to the thoroughfare, and only in stratum 6 was the relative density lower than in the thoroughfare (Table 1-17). Relative density of postlarvae in the thoroughfare in 1990 was either equivalent to or was more often greater in Cumberland Thoroughfare compared to strata along the river main-stem (Table 1-17). Postlarvae were captured in Cumberland Thoroughfare on 21 March 1990 indicating that spawning had occurred during the period of warm water temperature in mid-March.

Estimated relative densities of river herring postlarvae and gizzard shad postlarvae in 1989 and 1990 are presented in Table 1-18. River herring postlarvae appear to have been predominant in the study area in 1989 from early April to about 22 April, and relative density of postlarvae for each group appears to have been greatest during the interval from 22 April to about 11 May. Subsequent to 5 May the estimated relative density of river herring postlarvae was low. Similar results were found for the estimated relative density of river herring and gizzard shad postlarvae in 1990. Fig. 1-12 illustrates estimated

relative densities of river herring postlarvae showing similarities between the 1989 and 1990 sampling seasons.

Preflexion larvae were usually captured in all habitats during both sampling seasons (Table 1-19), but the distribution of postflexion larvae appeared to be more limited both temporally and spatially (Table 1-20). Relatively few postflexion larvae were captured in April of either sampling season. The highest relative density and broadest distribution of postflexion larvae is suggested to occur in late April-early May as demonstrated in 1990. High relative density observed on 18 May 1989 is suggested to have occurred after relative density had peaked as observed by the decline subsequent to 8 May, 1990. In April 1990, relative density of preflexion larvae appeared to increase faster in Cumberland Thoroughfare, Big Creek, and in the river main-stem than in Holts Creek, but from late April to mid-May the relative density appeared to have increased in Holts Creek and decreased in the remaining habitats. In April 1989, relative density of preflexion larvae was greater in Big Creek than in all other habitats, and was greatest in Holts Creek in mid-May 1989.

The percentage of clupeid postlarvae captured in all habitats progressively increased with time during the 1990 sampling season. Of the total mean relative density of clupeid postlarvae captured in 1990, approximately 3 percent was captured prior to 22 April, and 15 and 19 percent were captured on 22 April and 29 April, respectively. The bulk of herring postlarvae, 56 percent, were captured on 8 May and only 7 percent was captured on 12 May. In 1989, excluding larvae captured on 30 May, 39.3 percent of herring postlarvae were captured on 20 April and 60.7 percent were captured on 18 May. The results from the 1990 sampling season suggest that little information was lost from the 1989 season in early April.

Comparison of the distributions of herring postlarvae (pre and postflexion larvae) between potential habitats during the period from

mid-April to mid-May demonstrates a consistent pattern during the 1989 and 1990 sampling seasons (Table 1-21). Contingency table analysis demonstrated that the percentage of herring larvae captured in the potential habitats was not independent of the sampling date in April (X^2 =99.94, $p<0.001$), but that it was independent of sampling date in May (X^2 =11.40, $p>0.100$). Differences in the percentage distribution of postlarvae between potential habitats in April is suggested to be associated with an earlier movement of larvae into tidal creeks in 1989. However, by May during both seasons, the percentage distribution of postlarvae between potential habitats was markedly similar, despite differences in the magnitude of mean relative density observed between dates within and between years.

Mean standard length of herring larvae captured in the potential habitats during the 1989 sampling season differed significantly (Table 1-22). Significant interaction between the main-effects indicates that differences between habitats were related to the time of season supporting the hypothesis that the distribution of herring larvae shifts with ontogeny from the river main-stem into Holts Creek and Big Creek. Table 1-23 presents the mean and range of standard length of herring larvae captured in the potential habitats in 1989. Similar size ranges of larvae were observed in the 1990 samples, but a quantitative analysis was not conducted.

The distribution of postflexion herring larvae in Holts Creek appears to be characterized by pronounce diel variability (Table 1-20). Postflexion larvae were more common in collections made in Holts Creek earlier in the day in all samples obtained in late April (29 April) and May. Very few postflexion larvae were captured late in the day on 8 April and 22 April, but these were the only postflexion larvae captured at this time of the sampling season. The basis for this apparent behavior could not be investigated with the current sampling design.

DISCUSSION

Study of the early life histories of alewife and blueback herring is complicated by the coincident distribution of eggs and larvae of these species with eggs and larvae of gizzard shad, and the general morphological similarity of larvae of these groups. Prolarvae of river herring and gizzard shad can easily be identified based on the morphology of the yolk sac, but no method has been identified that can reliably delimit postlarvae of alewife and blueback herring.

A method to identify alewife and blueback herring was suggested by Chambers et al (1976) which was based on the number of myomeres between the dorsal fin insertion and the anal fin origin and on the ratio of the vent length to the standard length. Their conclusion, however, is not supported by other studies (Cianci 1969; Lam and Roff 1977; Bulak 1985). A study was conducted as part of this investigation to examine the methods suggested by Chambers et al. (1976) to delimit alewife and blueback herring postlarvae. Alewife and blueback herring larvae were reared from eggs of known parentage (see Appendices A and B), and comparison of these larvae demonstrated that morphometric and meristic characteristics are inadequate to delimit these species (see Appendix C). The results strongly suggest that characteristics identified by Chambers et al. (1976) apply to differences between river herring and gizzard shad, as documented by Lam and Roff (1977) and Bulak (1985).

Postlarvae of these species were not identified because of uncertainty in the taxonomic characteristics which delimit these groups. Because river herring prolarvae and gizzard shad prolarvae could be identified, the distributions of this stage for each of these groups were examined separately and all herring postlarvae were pooled. Eggs of river herring and gizzard shad could not be identified separately; therefore, it was not possible to document the distribution of spawning activity of these groups within the study area. Based on the relative densities (catch-per-effort) and distributions of river herring and

gizzard shad prolarvae, early life stages of these species appear to be coincident throughout the tidal freshwater reach of the Pamunkey River. Warmer temperature in 1990 appears to have initiated spawning earlier compared to 1989.

The consistent capture of herring eggs at high relative densities in Cumberland Thoroughfare compared to the river main-stem, especially in April, suggests that the thoroughfare is an important area for spawning by river herring and possibly by gizzard shad. Spawning in the thoroughfare may occur as a result of herring being attracted into this zone by tidal currents which flow through the thoroughfare at comparatively high velocity due to a funneling-effect. Alternatively, eggs occurring in the thoroughfare may not be dispersed to the extent that would be expected in the river main-stem.

Relatively few herring eggs were captured in the five potential habitats, most likely because eggs of these groups tend to be adhesive, to varying degree, and range in buoyancy from semi-demersal to pelagic. Low relative densities of eggs captured by the pushnet suggests that relatively few eggs may have been entrained in near-surface water. Consequently, eggs sampled using the pushnet may not provide an accurate estimate of egg densities and may underestimate the use of these areas by herring for spawning. The relative densities of herring eggs in Cumberland Thoroughfare was much higher in samples taken by oblique tow through the water column compared to samples taken in the near-surface water. Birdsong et al. (1987) reported higher densities of herring eggs in bottom water compared to surface in the non-tidal reach of the Pamunkey River. During their study, peak egg densities occurred during the first week of May, and higher concentrations of eggs were reported in samples from the river compared to samples at various locations within the adjacent Crump/Pollard creek system.

Higher relative densities of river herring and gizzard shad prolarvae in the tidal creeks during April suggests that these areas may

be used for spawning, although advection may transport prolarvae into these areas. The relative densities of river herring prolarvae in the tidal creeks appears to decline from April to May at about the time the relative density of gizzard shad increases. Lower relative densities of river herring prolarvae in May suggests that most spawning occurred during April; however, lower relative density of river herring prolarvae in Cumberland Thoroughfare and in the tidal creeks in May compared to April may indicate a shift in spawning site selection out of the study area. Higher relative densities of gizzard shad prolarvae in tidal creeks may indicate that this species utilized these areas for spawning to a greater extent than do river herring, but this conclusion is speculative.

Tidal freshwater marshes and associated tidal creeks are significant nursery areas for fishes (Paller 1987; Rozas and Odum 1987a, b; Rozas et al. 1988; Killgore et al. 1989). Higher densities of larval and juvenile stages of many species are found in habitats adjacent to the main channel in tidal or non-tidal and freshwater or estuarine ecosystems (Hess and Winger 1976; Chubb and Liston 1986; Paller 1987; Rozas and Odum 1987a; Thayer and Chester 1989; Scott and Nielsen 1989). The tidal creeks studied for this investigation appear to be important nursery habitat for herring postlarvae, especially Holts Creek. Preflexion larvae appear to recruit to tidal creeks from the river and the thoroughfare and, as larvae increased in size and age, they appeared to remain preferentially in Holts Creek, possibly due to higher concentrations of potential prey (see Part II). The relatively narrow and shallow character of Holts Creek may provide refuge from predators compared to Big Creek, the thoroughfare, and the river main-stem.

An important factor not considered by this study is the longitudinal distribution of herring postlarvae within the two creeks, and the possibility that the sampling site in Big Creek was not at the same relative location compared to the sampling site in Holts Creek.

Rozas and Odum (1987a) reported no significant difference in the total numbers of fishes captured in the headwater and main creek zones in a tidal freshwater marsh of the Chickahominy River, Virginia. Their investigation, however, was conducted from July through October, well after the spawning season of anadromous herring, and focused on juvenile fishes and crustaceans.

Herring postlarvae appear to use tidal creeks for a relatively short time period, the most important period occurring from about late April to mid-May. Dissimilar distributions of larvae in late April between the two sampling seasons followed relatively shortly thereafter in early to mid-May by very similar partitioning between habitats suggests that movement into near-shore areas or tidal creeks occurs over a short period of time. By the end of May, 1989, relatively few postlarvae occurred in the tidal creeks which suggests a short residence time for postlarvae in these creeks. This apparent short residence time may also be associated with increased gear avoidance by older, larger postlarvae. The significance of these tidal creeks for older postlarvae and juveniles, and the general applicability of these results for other creeks in the tidal freshwater reach of the Pamunkey River is unknown. Loesch et al. (1982) reported that juvenile *Alosa* are lower in the water column during the day, but the extent of lateral movements between the channel and near-shore zones or marshes is unknown.

Postflexion larvae appear to exhibit diel variation in their distribution within Holts Creek, but this apparent behavior could not be validated for this study. Possible explanations might include alteration in depth distribution or longitudinal distribution in the creek in relation to tidal phase, or alteration in depth distribution associated with inflation/deflation of the swimbladder. Concentration of larvae within the banks of Holts Creek may be increased during periods of low water during the tide cycle, while dispersal may be increased during periods of high water. Alternatively, prolonged

shading of Holts Creek by the adjacent forest canopy might influence the behavior of herring postlarvae. Holts Creek remains shaded longer during the day compared to Big Creek or the river which may maintain relatively lower light conditions in the water column. Postlarvae may remain for a longer time in the near-surface water where they were subject to capture with the pushnet. Further study of distributions of herring postlarvae over short time intervals will be necessary to further understand the behavior of this life stage in the tidal freshwater marsh ecosystem.

LITERATURE CITED

Anderson, J. T. 1988. A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. Journal of Northwest Atlantic Fishery Science 8: 55-56.

Anon. 1989a. Virginia's environmental agenda of the 1990's. The Virginia Environmental Assembly. 66 p.

Anon. 1989b. Local climatological data, monthly summary, Richmond, Virginia: March. National Oceanic and Atmospheric Administration, National Weather Service. 4 p.

Anon. 1989c. Local climatological data, monthly summary, Richmond, Virginia: April. National Oceanic and Atmospheric Administration, National Weather Service. 4 p.

Anon. 1989d. Local climatological data, monthly summary, Richmond, Virginia: May. National Oceanic and Atmospheric Administration, National Weather Service. 4 p.

Anon. 1990a. Local climatological data, monthly summary, Richmond, Virginia: March. National Oceanic and Atmospheric Administration, National Weather Service. 4 p.

Anon. 1990b. Local climatological data, monthly summary, Richmond, Virginia: April. National Oceanic and Atmospheric Administration, National Weather Service. 4 p.

Anon. 1990c. Local climatological data, monthly summary, Richmond, Virginia: May. National Oceanic and Atmospheric Administration, National Weather Service. 4 p.

Birdsong, R. S., D. E. Peterson, and D. W. Byrd. 1987. A survey of alosine fish occurrence and spawning utilization of the Crump/Pollard creek system and the adjacent Pamunkey River in Hanover County, Virginia. Final Report, Old Dominion University Research Foundation, Norfolk, Virginia. 8 p.

Brooks, T. J. 1983. Pamunkey River slack water data report; temperature, salinity, dissolved oxygen: 1970-1980. Data Report Number 20. Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia. 40 p.

Bulak, J. S. 1985. Distinction of larval blueback herring, gizzard shad, and threadfin shad from the Santee-Cooper drainage, South Carolina. Journal of the Elisha Mitchell Scientific Society 101: 177-186.

Cianci, J. M. 1969. Larval development of the alewife *Alosa pseudoharengus* Wilson, and the glut herring, *Alosa aestivalis* Mitchill. M.S. Thesis, University of Connecticut, Storrs, Connecticut. 62 p.

Chambers, J. R., J. A. Musick, and J. Davis. 1976. Methods of distinguishing larval alewife from blueback herring. Chesapeake Science 17: 93-100.

Chubb, S. L. and C. R. Liston. 1986. Density and distribution of larval fishes in Pentwater Marsh, a coastal wetland on Lake Michigan. Journal of Great Lakes Research 12: 332-343.

Crecco, V. A. and T. Savoy. 1987a. Review of recruitment mechanisms of the American shad: the critical period and match-mismatch hypotheses reexamined. American Fisheries Society Symposium 1: 455-468.

Crecco, V. and T. Savoy. 1987b. Effects of climatic and density-dependent factors on intra-annual mortality of larval American shad. American Fisheries Society Symposium 2: 69-81.

Hess, T. B. and P. V. Winger. 1976. The occurrence and distribution of larval fish in the Cumberland River. Proceedings of the Annual Conference of the Southeastern Association of Game and Fish Commissions 30: 295-310.

Killgore, K. J., R. P. Morgan II, and N. B. Rybicki. 1989. Distribution and abundance of fishes associated with submersed aquatic plants in the Potomac River. North American Journal of Fisheries Management 9: 101-111.

Lam, C. N. H., and J. C. Roff. 1977. A method for separating alewife *Alosa pseudoharengus* from gizzard shad *Dorosoma cepedianum* larvae. Journal of Great Lakes Research 3: 313-316.

Leggett, W. C. 1977. Density dependence, density independence, and recruitment in the American shad (*Alosa sapidissima*) population of the Connecticut River, pages 3-17. *In*: W. Van Winkle (editor), Assessing the effects of power-plant-induced mortality on fish populations. Pergamon Press, New York. 380 p.

Loesch, J. G., W. H. Kriete, and E. J. Foell. 1982. Effects of light intensity on the catchability of juvenile anadromous *Alosa* species. Transactions of the American Fisheries Society 111: 41-44.

Markle, D. F. 1984. Phosphate buffered formalin for long term preservation of formalin fixed ichthyoplankton. Copeia 1984: 525-528.

May, R. C. 1974. Larval mortality in marine fishes and the critical period concept, pages 3-19. *In*: J. H. S. Blaxter (editor), The early life history of fish. Springer-Verlag, Berlin.

Odum, W. E., T. J. Smith III, J. K. Hoover, and C. C. McIvor. 1984. The ecology of tidal freshwater marshes of the United States east coast: a community profile. U. S. Fish and Wildlife Service, FWS/OBS-83/17. 177 pp.

Paller, M. H. 1987. Distribution of larval fish between macrophyte beds and open channels in a southeastern floodplain swamp. Journal of Freshwater Ecology 4: 191-200.

Prugh, B. J., Jr., F. J. Easton, and D. L. Belval. 1990. Water resources data, Virginia: Water year, 1989. U.S. Geological Survey, Water-data Report VA-89-1.

Prugh, B. J., Jr., F. J. Easton, and D. L. Belval. 1991. Water resources data, Virginia: Water year, 1990. U.S. Geological Survey, Water-data Report VA-90-1.

Rozas, L. P. and W. E. Odum. 1987a. Use of tidal freshwater marshes by fishes and macrofaunal crustaceans along a marsh stream-order gradient. Estuaries 10: 36-43.

Rozas, L. P. and W. E. Odum. 1987b. Fish and macrocrustacean use of submerged plant beds in tidal freshwater marsh creeks. Marine Ecology, Progress Series 38: 101-108.

Rozas, L. P., C. C. McIvor, and W. E. Odum. 1988. Intertidal rivulets and creekbanks: corridors between tidal creeks and marshes. Marine Ecology, Progress Series 47: 303-307.

Scott, M. T. and L. A. Nielsen. 1989. Young fish distribution in backwaters and main-channel borders of the Kanawha River, West Virginia. Journal of Fish Biology 35 (Suppl. A): 21-27.

Thayer, G. W. and A. J. Chester. 1989. Distribution and abundance of fishes among basin and channel habitats in Florida Bay. Bulletin of Marine Science 44: 200-219.

Tranter, D. J. and P. E. Smith. 1968. Filtration performance, pages 27-56. *In*: D. J. Tranter (editor), Reviews on zooplankton sampling methods. UNESCO Monographs on Oceanographic Methodology No. 2, Geneva, Switzerland.

Table 1-1. Mean monthly values for daily air temperature (measured at Byrd International Airport, Richmond, Virginia), daily water temperature (lower York River measured at the Virginia Institute of Marine Science), and daily river flow (U.S. Geologic Service, National Stream-quality Accounting Network, Station 01673000 located near Hanover, Virginia) in March, April, and May of the 1989-1990 sampling seasons.

Month	Year	
	1989	1990
Mean daily air temperature (C)		
March	8.8	11.2
April	13.2	14.4
May	17.8	18.8
Mean	13.3	14.8
Mean daily water temperature (C)		
March	6.6	10.6
April	13.2	13.7
May	19.0	19.1
Mean	12.9	14.5
Mean daily Pamunkey River flow (cfs)		
March	2243	1262
April	1258	1970
May	2634	2303
Mean	2022	1860

Table 1-2. Mean monthly values for water temperature and dissolved oxygen measured in tidal freshwater of the Pamunkey River during sampling events in March, April, and May of the 1989-1990 sampling seasons. Values in parentheses indicated the number of sampling events in each month during which these variables were measured.

Month	Year	
	1989	1990
Mean water temperature (C)		
March	10.9 (1)	14.3 (1)
April	15.7 (8)	17.9 (2)
May	19.3 (6)	21.5 (1)
Mean	15.3	17.9
Mean dissolved oxygen (mg L^{-1})		
March	9.0 (1)	8.6 (1)
April	8.2 (7)	7.1 (2)
May	6.9 (6)	8.1 (1)
Mean	8.0	7.9

Table 1-3. Water temperature (C) in potential tidal freshwater habitats of the Pamunkey River during the 1989-1990 sampling seasons.

Location	Date			Mean
	1989			
	April	May		
	20	18	30	
Holts Creek, early	15.0	17.0	23.5	18.5
Holts Creek, late	-	-	26.0	
Big Creek	20.0	18.0	22.5	20.2
Channel	21.0	17.0	24.5	20.8
Flats	20.5	18.0	25.0	21.2
Thoroughfare	20.5	17.0	24.5	20.8
Mean	19.4	17.4	24.3	

	1990							
	March	April				May		
	17	8	16	22	29	8	12	
Holts Creek, early	18.0	9.5	16.5	17.5	21.0	19.0	18.0	17.1
Holts Creek, late	-	13.0	16.0	21.0	22.5	24.0	20.0	19.4
Big Creek	19.0	10.5	17.0	15.5	22.0	20.0	18.5	17.5
Channel	18.5	12.0	15.0	16.0	22.5	21.0	20.0	17.9
Flats	18.5	12.0	15.0	17.0	22.5	21.5	20.0	17.2
Thoroughfare	18.0	13.5	15.0	17.5	21.5	21.0	20.0	18.1
Mean	18.4	11.8	15.8	17.4	22.0	21.1	19.5	

Table 1-4. Dissolved oxygen concentrations (mg L^{-1}) in the Pamunkey River and in Cumberland Thoroughfare during the 1989-1990 sampling seasons. Values in parentheses indicate the stratum of the corresponding interval along the river channel.

Location	Date													Mean
	1989													
	March	April						May						
	28	4	6	12	18	22	28	3	5	11	16	20	25	
P 45-47 (1)	-	8.5	8.0	8.8	8.8	8.0	8.2	7.1	6.8	7.3	6.8	7.4	7.4	7.6
P 48-50 (2)	9.4	8.5	8.0	8.8	8.8	7.8	8.2	7.3	6.7	7.1	6.9	7.3	7.5	7.9
P 51-53 (3)	9.2	8.4	8.0	9.1	8.6	7.5	7.8	7.9	6.4	6.6	6.8	7.5	6.9	7.7
P 54-56 (4)	9.0	8.3	6.2	9.1	8.4	7.4	7.7	6.3	6.8	7.3	6.8	7.6	5.7	7.4
P 57-59 (5)	9.2	-	-	9.1	8.2	7.3	7.0	5.9	6.9	6.6	6.9	7.4	5.6	7.3
P 60-62 (6)	8.4	-	-	9.2	8.2	-	7.1	5.8	7.0	6.7	7.5	7.1	5.6	7.3
Thoroughfare	-	8.6	-	8.2	8.6	7.6	8.2	7.1	6.7	7.1	6.9	7.3	6.9	7.6
Mean	9.0	8.5	7.6	8.9	8.5	7.6	7.7	6.8	6.8	7.0	6.9	7.4	6.5	

Location	1990			Mean
	March	April	May	
	21	27	9	
P 45-47 (1)	9.0	8.2	9.2	8.8
P 48-50 (2)	8.9	8.1	9.2	8.7
P 51-53 (3)	8.8	7.4	8.9	8.4
P 54-56 (4)	8.7	6.7	7.7	7.7
P 57-59 (5)	8.2	6.2	7.0	7.1
P 60-62 (6)	8.1	6.2	6.8	7.0
Thoroughfare	8.9	7.3	9.0	8.4
Mean	8.7	7.2	8.3	

Table 1-5. Dissolved oxygen concentrations (mg L^{-1}) in potential tidal freshwater habitats of the Pamunkey River during the 1989-1990 sampling seasons.

Location	Date							Mean
	1989							
	April			May				
	20			18			30	
Holts Creek, early	6.3			6.2			6.5	6.3
Holts Creek, late	-			-			8.2	
Big Creek	8.1			7.7			6.0	7.3
Channel	7.9			7.7			7.6	7.7
Flats	8.2			7.6			7.8	7.9
Thoroughfare	8.1			7.2			8.3	7.9
Mean	7.7			7.3			7.4	
	1990							
	March	April				May		
	17	8	16	22	29	8	12	
Holts Creek, early	7.8	9.1	8.1	8.7	4.8	6.1	7.2	7.4
Holts Creek, late	-	8.7	8.4	8.9	6.4	9.2	8.5	8.4
Big Creek	8.4	8.9	9.0	8.3	7.2	8.0	8.1	8.3
Channel	8.4	8.8	8.2	8.1	5.6	7.2	7.2	7.6
Flats	8.3	8.9	8.1	8.4	6.2	7.6	8.1	7.9
Thoroughfare	8.6	8.6	8.5	8.7	7.0	8.0	8.3	8.2
Mean	8.3	8.8	8.4	8.5	6.2	7.7	7.9	

Table 1-6. Water transparency (cm) in potential tidal freshwater habitats of the Pamunkey River during the 1989-1990 sampling seasons.

Location	Date							Mean
	1989							
	April			May				
	20			18		30		
Holts Creek, early	40			37		55		44
Holts Creek, late	-			-		52		
Big Creek	55			48		50		51
Channel	62			50		56		56
Flats	50			54		48		51
Thoroughfare	54			50		43		49
Mean	52			48		51		
	1990							
	March	April				May		
	17	8	16	22	29	8	12	
Holts Creek, early	30	40	38	45	68	52	35	44
Holts Creek, late	-	40	43	45	54	38	31	42
Big Creek	55	40	40	50	52	47	38	46
Channel	50	65	65	45	56	56	30	52
Flats	30	42	40	50	32	53	50	42
Thoroughfare	35	40	47	49	50	50	31	43
Mean	40	45	46	47	52	49	36	

Table 1-7. Relative density (100 m^3) of herring eggs in the Pamunkey River and in Cumberland Thoroughfare during the 1989-1990 sampling seasons. Values in parentheses indicate the stratum number of the corresponding interval along the river channel.

Location	Date												Mean
	1989												
	April						May						
	4	12	18	22	25	28	3	5	11	16	20	25	
P 45-47 (1)	194	0	0	396	16	0	12	0	0	0	0	0	52
P 48-50 (2)	4	0	24	0	0	0	0	0	0	0	0	0	2
P 51-53 (3)	11	0	0	0	10	0	0	0	0	0	0	0	2
P 54-56 (4)	7	0	0	0	0	0	0	0	0	0	0	0	<1
P 57-59 (5)	0	0	0	0	0	0	26	7	0	62	0	0	8
P 60-62 (6)	0	208	0	0	0	0	9	142	0	7	0	0	31
Thoroughfare	84	37	1523	1007	64	0	0	241	0	0	0	0	246
Mean	42	35	221	220	13	0	7	56	0	10	0	0	

Location	1990			Mean
	March	April	May	
	21	10	27	9	
P 45-47 (1)	0	31	23	0	14
P 48-50 (2)	0	0	61	0	15
P 51-53 (3)	0	0	3	0	<1
P 54-56 (4)	0	0	3	0	4
P 57-59 (5)	0	301	39	0	85
P 60-62 (6)	0	220	24	0	61
Thoroughfare	43	161	917	0	280
Mean	6	102	158	0	

Table 1-8. Relative density (100 m^3) of herring eggs in potential tidal freshwater habitats of the Pamunkey River, Virginia, during the 1989-1990 sampling seasons.

Location	Date							Mean
	1989							
	April			May				
	20			18		30		
Holts Creek, early	0			0		7		2
Holts Creek, late	-			-		48		
Big Creek	14			0		0		4
Channel	0			0		0		0
Flats	0			0		0		0
Thoroughfare	11			3		173		62
Mean	5			<1		38		
	1990							
	March	April				May		
	17	8	16	22	29	8	12	
Holts Creek, early	0	0	0	0	4	0	0	<1
Holts Creek, late	-	0	0	0	0	0	0	0
Big Creek	0	0	0	2	3	0	0	<1
Channel	0	0	2	0	0	0	0	<1
Flats	0	0	3	0	0	0	0	<1
Thoroughfare	0	30	3	10	0	0	0	6
Mean	0	5	1	2	1	0	0	

Table 1-9. Relative density (100 m^{-3}) of river herring prolarvae in the Pamunkey River and in Cumberland Thoroughfare during the 1989-1990 sampling seasons. Values in parentheses indicate the stratum of the corresponding interval along the river channel.

Location	Date												Mean
	1989												
	April						May						
	4	12	18	22	25	28	3	5	11	16	20	25	
P45-47 (1)	53	452	61	684	140	122	11	37	29	0	0	0	132
P48-50 (2)	129	170	149	248	422	71	26	16	94	0	0	0	110
P51-53 (3)	22	250	75	120	261	144	6	34	218	0	0	5	95
P54-56 (4)	15	26	7	6	209	42	176	10	87	0	15	5	50
P57-59 (5)	53	45	170	396	73	63	314	51	32	15	102	0	110
P60-62 (6)	62	0	446	684	1374	0	2017	10	0	21	22	3	387
Thoroughfare	118	130	263	439	139	147	0	60	78	0	7	0	115
Mean	65	153	167	368	374	84	364	31	77	5	21	2	

	1990			
	March	April		May
	21	10	27	9
P45-47 (1)	25	0	0	0	6
P48-50 (2)	0	70	12	0	21
P51-53 (3)	23	47	6	0	19
P54-56 (4)	12	18	0	0	8
P57-59 (5)	0	64	26	30	30
P60-62 (6)	10	98	278	153	134
Thoroughfare	24	40	46	0	28
Mean	13	48	53	26	

Table 1-10. Relative densities of river herring prolarvae in Cumberland Thoroughfare compared to strata along the Pamunkey River main-stem channel during the 1989-1990 sampling seasons. Values in parentheses indicate the stratum of the corresponding interval along the river channel. Table values indicate the number of sampling events in which prolarva relative densities in Cumberland Thoroughfare were greater (G), lower (L), or equivalent (E) to prolarva relative densities in strata of the river channel. An asterisk indicates collections in which no prolarvae were captured in either location.

Location	Year					
	1989			1990		
	G	L	E	G	L	E
P45-47 (1)	6	4	2*	2	1	1*
P48-50 (2)	5	5	2*	2	1	1*
P51-53 (3)	5	6	1*	2	1	1*
P54-56 (4)	6	5	1*	3	0	1*
P57-59 (5)	8	3	1*	2	2	0
P60-62 (6)	5	7	0	1	3	0

Table 1-11. Relative densities of river herring prolarvae in Cumberland Thoroughfare compared to strata along the Pamunkey River main-stem channel during April and May, 1989. Values in parentheses indicate the stratum of the corresponding interval along the river channel. Table values indicate the number of sampling events in which prolarva relative densities in Cumberland Thoroughfare were greater (G), lower (L), or equivalent (E) to prolarva relative densities in strata of the river channel. An asterisk indicates collections in which no prolarvae were captured in either location.

Location	Month					
	April			May		
	G	L	E	G	L	E
P45-47 (1)	3	3	0	3	1	2*
P48-50 (2)	3	3	0	2	2	2*
P51-53 (3)	3	3	0	2	3	1*
P54-56 (4)	5	1	0	1	4	1*
P57-59 (5)	6	0	0	2	3	1*
P60-62 (6)	3	3	0	2	4	0

Table 1-12. Relative density (100 m^3) of river herring prolarvae in potential tidal freshwater habitats of the Pamunkey River, Virginia, during the 1989-1990 sampling seasons.

Location	Date			Mean
	1989			
	April	May		
	20	18	30	
Holts Creek, early	0	3	0	1
Holts Creek, late	-	-	0	
Big Creek	18	0	0	6
Channel	3	0	0	1
Flats	6	0	0	2
Thoroughfare	0	0	0	0
Mean	5	1	0	

	1990							
	March	April				May		
	17	8	16	22	29	8	12	
Holts Creek, early	8	2	5	169	4	0	3	27
Holts Creek, late	-	9	14	0	47	3	9	14
Big Creek	26	9	5	55	115	0	0	30
Channel	1	4	2	30	67	0	4	15
Flats	20	2	171	17	61	9	0	40
Thoroughfare	10	18	18	295	250	0	0	84
Mean	13	7	36	94	91	2	3	

Table 1-13. Relative density (100 m^3) of gizzard shad prolarvae in the Pamunkey River and in Cumberland Thoroughfare during the 1989-1990 sampling seasons. Values in parentheses indicate the stratum of the corresponding interval along the river channel.

Location	Date												Mean
	1989												
	April						May						
	4	12	18	22	25	28	3	5	11	16	20	25	
P45-47 (1)	0	0	0	12	62	0	63	37	0	29	38	88	27
P48-50 (2)	0	0	0	37	343	92	68	41	55	92	82	104	76
P51-53 (3)	0	0	0	0	136	431	26	299	18	21	147	124	100
P54-56 (4)	0	0	0	7	209	217	743	185	0	0	148	520	169
P57-59 (5)	0	0	0	6	10	25	15	102	0	108	31	245	57
P60-62 (6)	0	0	0	13	62	0	37	10	0	0	32	277	36
Thoroughfare	0	0	0	181	225	457	66	45	131	67	64	334	131
Mean	0	0	0	37	150	175	165	103	29	45	77	242	

	1990			
	March	April		May	
	21	10	27	9	
P45-47 (1)	0	0	4	0	1
P48-50 (2)	0	7	16	15	10
P51-53 (3)	0	0	194	0	49
P54-56 (4)	0	0	41	34	19
P57-59 (5)	0	0	204	16	55
P60-62 (6)	0	0	458	255	178
Thoroughfare	5	0	279	0	71
Mean	1	1	171	46	

Table 1-14. Relative densities of gizzard shad prolarvae in Cumberland Thoroughfare compared to strata along the Pamunkey River main-stem channel during the 1989-1990 sampling seasons. Values in parentheses indicate the stratum of the corresponding interval along the river channel. Table values indicate the number of sampling events in which prolarva relative densities in Cumberland Thoroughfare were greater (G), lower (L), or equivalent (E) to prolarva relative densities in strata of the river channel. An asterisk indicates collections in which no prolarvae were captured in either location.

Location	Year					
	1989			1990		
	G	L	E	G	L	E
P45-47 (1)	8	1	3*	2	0	2*
P48-50 (2)	6	3	3*	2	2	0
P51-53 (3)	7	2	3*	2	0	2*
P54-56 (4)	5	4	3*	2	1	1*
P57-59 (5)	6	3	3*	2	1	1*
P60-62 (6)	9	0	3*	1	2	1*

Table 1-15. Relative density (100 m^3) of gizzard shad prolarvae in potential tidal freshwater habitats of the Pamunkey River, Virginia, during the 1989-1990 sampling seasons.

Location	Date			Mean
	1989			
	April	May		
	20	18	30	
Holts Creek, early	19	57	125	67
Holts Creek, late	-	-	28	
Big Creek	10	28	145	61
Channel	0	11	34	15
Flats	0	25	3	9
Thoroughfare	0	19	105	41
Mean	6	28	73	

	1990							
	March	April				May		
	17	8	16	22	29	8	12	
Holts Creek, early	0	0	3	41	289	81	186	86
Holts Creek, late	-	0	0	0	144	166	139	75
Big Creek	11	0	0	15	476	3	68	82
Channel	0	0	0	0	100	0	14	16
Flats	0	0	0	7	180	54	21	37
Thoroughfare	0	0	0	144	63	0	67	39
Mean	2	0	1	35	209	51	83	

Table 1-16. Relative density (100 m^{-3}) of herring postlarvae in the Pamunkey River and in Cumberland Thoroughfare during the 1989-1990 sampling seasons. Values in parentheses indicate the stratum of the corresponding interval along the river channel.

Location	Date												Mean
	1989												
	April						May						
	4	12	18	22	25	28	3	5	11	16	20	25	
P45-47 (1)	31	312	18	72	93	90	322	261	97	52	5	6	113
P48-50 (2)	35	175	197	37	131	751	1802	94	75	123	4	18	287
P51-53 (3)	22	59	22	48	125	99	681	1378	0	21	4	11	206
P54-56 (4)	56	0	10	247	218	300	4646	278	0	69	8	77	492
P57-59 (5)	18	0	12	252	0	504	1046	96	0	46	24	210	184
P60-62 (6)	0	0	10	27	21	309	954	0	0	7	0	124	121
Thoroughfare	84	19	49	26	129	16	822	151	79	14	0	46	120
Mean	35	81	45	101	102	296	1468	323	40	47	6	70	

	1990			
	March	April		May
	21	10	27	9
P45-47 (1)	15	39	73	0	32
P48-50 (2)	0	25	44	0	17
P51-53 (3)	0	36	143	9	47
P54-56 (4)	0	18	129	45	48
P57-59 (5)	0	0	1519	95	404
P60-62 (6)	5	0	446	61	128
Thoroughfare	38	40	151	17	62
Mean	8	23	358	32	

Table 1-17. Relative densities of herring postlarvae in Cumberland Thoroughfare compared to strata along the Pamunkey River main-stem channel during the 1989-1990 sampling seasons. Values in parentheses indicate the stratum of the corresponding interval along the river channel. Table values indicate the number of sampling events in which postlarva relative densities in Cumberland Thoroughfare were greater (G), lower (L), or equivalent (E) to prolarva relative densities in strata of the river channel. An asterisk indicates collections in which no prolarvae were captured in either location.

Location	Year					
	1989			1990		
	G	L	E	G	L	E
P45-47 (1)	8	7	0	4	0	0
P48-50 (2)	4	8	0	4	0	0
P51-53 (3)	6	6	0	4	0	0
P54-56 (4)	4	8	0	3	1	0
P57-59 (5)	6	6	0	2	2	0
P60-62 (6)	7	4	0	2	2	0

Table 1-18. Estimated relative densities of river herring (*Alosa* spp.) and gizzard shad (*D. cepedianum*) postlarvae captured during the 1989-1990 sampling seasons.

Date	Percent Composition of herring prolarvae		Relative Density of herring postlarvae	Estimated Relative Density of postlarvae by taxon	
	Alosa spp.	*D. cepedianum*		*Alosa* spp.	*D. cepedianum*
			1989		
22 March	0	0	0	0	0
28 March	100.0	0	2	2	0
4 April	100.0	0	83	83	0
6 April	100.0	0	188	188	0
12 April	100.0	0	248	248	0
14 April	99.4	0.6	272	270	2
18 April	100.0	0	197	197	0
22 April	90.3	9.7	529	478	1
25 April	75.1	4.9	649	487	162
28 April	36.6	3.4	545	199	346
3 May	70.1	9.9	2182	1530	652
5 May	17.9	2.1	489	88	401
11 May	86.2	3.8	117	101	16
16 May	12.8	7.2	101	13	88
20 May	22.5	7.5	110	25	85
25 May	1.1	8.9	302	3	299
			1990		
17 March	100.0	0	15	15	0
10 April	97.9	2.1	71	70	1
27 April	26.1	73.9	599	156	443
9 May	36.5	63.5	119	43	76

Table 1-21. Percentage composition of herring postlarvae between potential tidal freshwater habitats in mid- to late April and early to mid-May during the 1989-1990 sampling seasons. (Holts Creek samples from 1989 were collected early in the day; therefore, samples collected from Holts Creek early in the day in 1990 were used for comparison.)

Location	Year		
	1989	1990	
	20 April	22 April	29 April
Holts Creek	31	1	12
Big Creek	42	56	17
Channel	8	17	16
Flats	3	13	43
Thoroughfare	16	13	13
	18 May	8 May	12 May
Holts Creek	80	77	78
Big Creek	13	16	12
Channel	1	1	1
Flats	0	5	2
Thoroughfare	6	1	7

Table 1-22. Analysis of variance of standard length of herring larvae captured in potential tidal freshwater habitats during the 1989 sampling season.

Source	df	SS	MS	F	F prob.
Time of season	2	119.596	59.798	15.414	<0.001
Habitat	4	128.625	32.156	8.289	<0.000
Interaction	8	101.267	12.658	3.263	0.001
Explained	14	384.448	27.461	7.078	<0.001
Residual	609	2362.578	3.879		
Total	623	2747.026	4.409		

Table 1-23. Unadjusted catch and standard length (mean and range) of river herring prolarvae and herring postlarvae captured in potential habitats during the 1989 sampling season.

Habitat	Catch (Unadjusted)	Standard length		
		Mean	Minimum	Maximum
Holts Creek	383	6.4	3.0	15.9
Big Creek	142	5.7	3.1	15.0
Channel	26	4.9	3.0	6.2
Flats	23	4.9	2.8	6.8
Thoroughfare	50	5.1	2.9	5.9

Figure 1-1. Location of the Pamunkey River in Virginia.

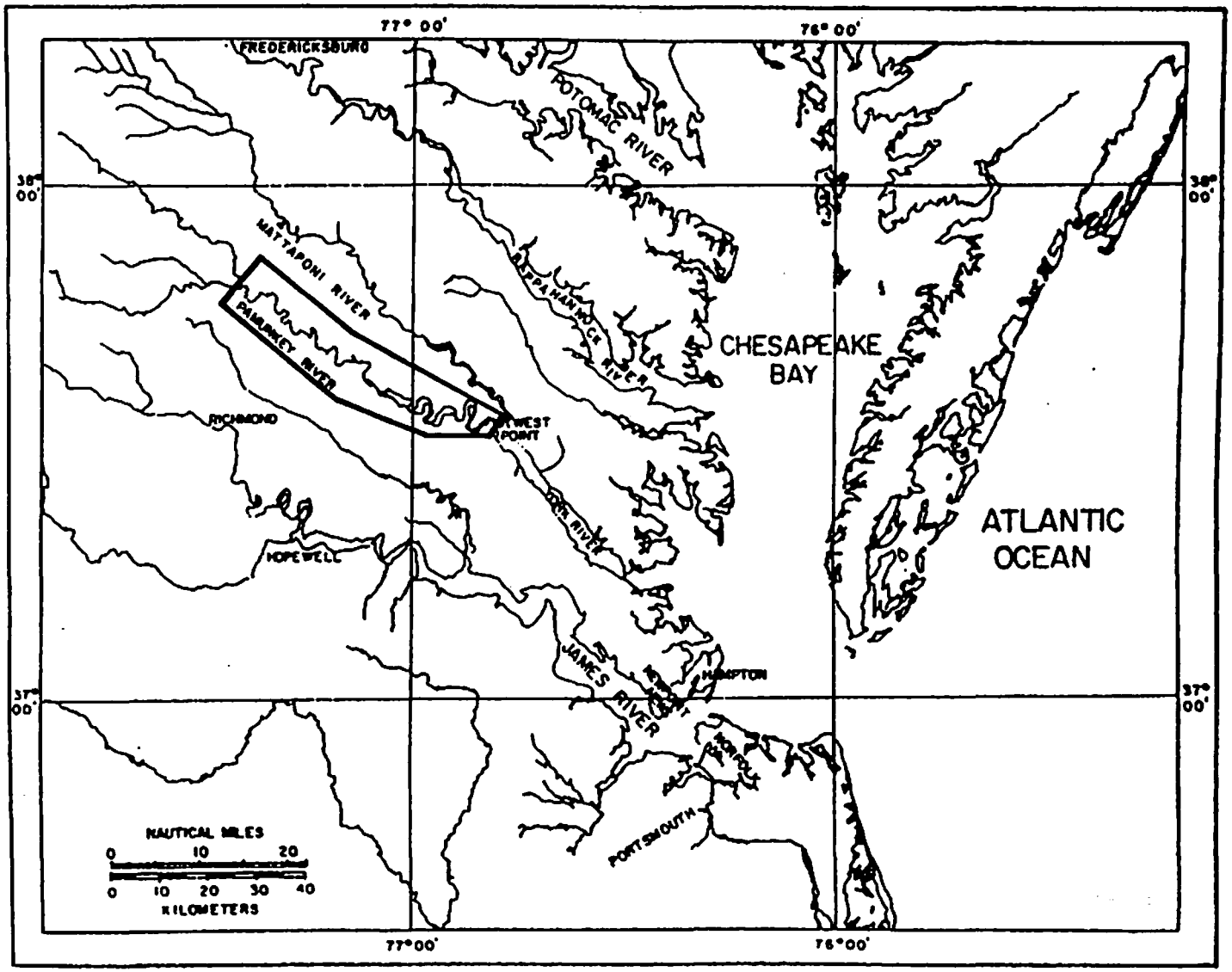

77° 00'
76° 00'
FREDERICKSBURG
POTOMAC RIVER
38° 00'
MATTAPONI RIVER
PAMUNKEY RIVER
RAPPAHANNOCK RIVER
CHESAPEAKE BAY
WEST POINT
RICHMOND
YORK RIVER
ATLANTIC OCEAN
HOPEWELL
JAMES RIVER
HAMPTON
37° 00'
PORTSMOUTH
NAUTICAL MILES
0 10 20
0 10 20 30 40
KILOMETERS
77°00'
76°00'

Figure 1-2. Study area in the tidal freshwater reach of the Pamunkey River from mile (nautical) P-45 to P-62, inclusive. The boxed area highlights the location of the Cumberland Thoroughfare region illustrated in Figure 1-3a.

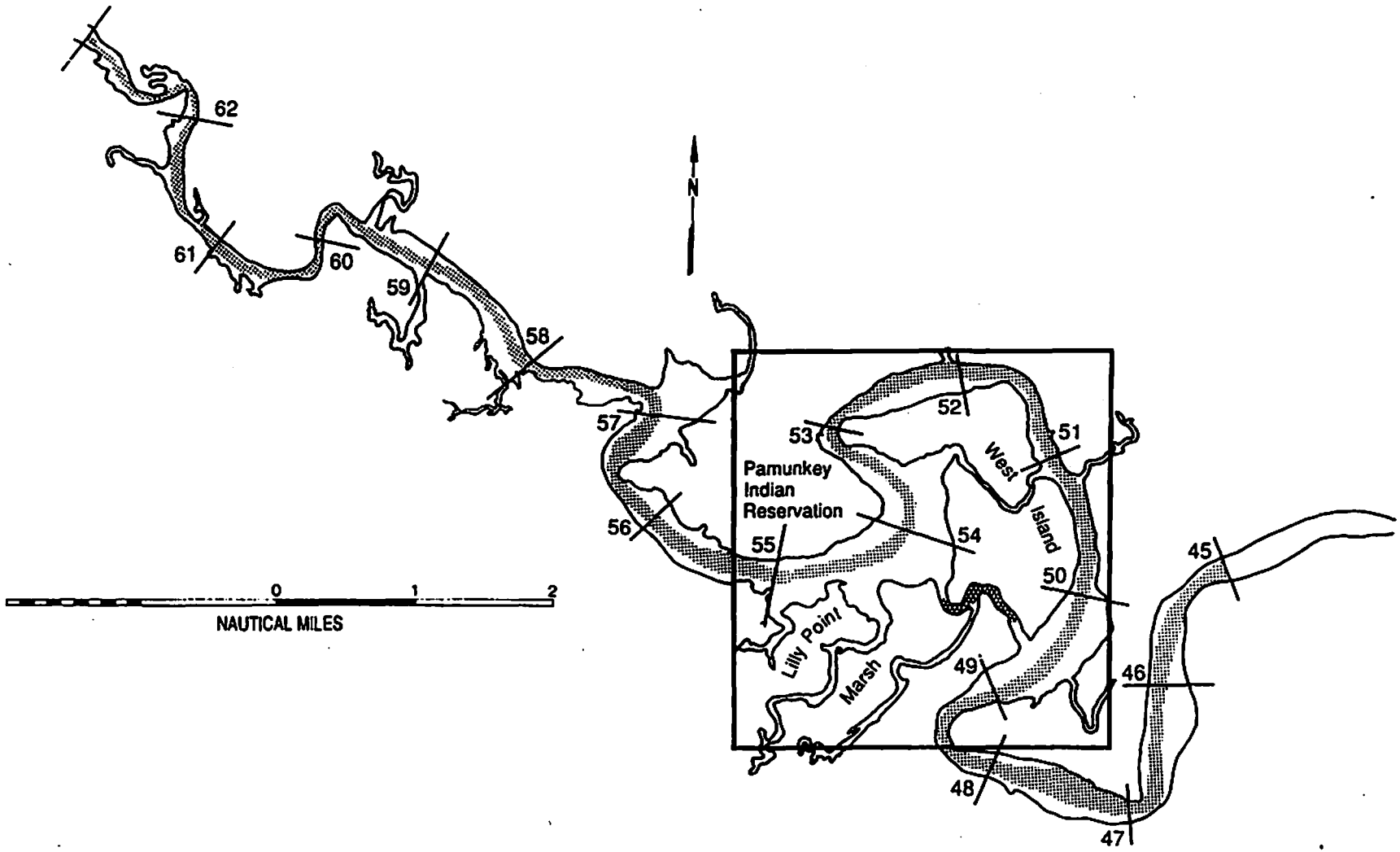

62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
N
Pamunkey
Indian
Reservation
West
Island
Lilly Point
Marsh
0
1
2
NAUTICAL MILES

Figure 1-3a. Study area in the Cumberland Thoroughfare region showing sampling locations within potential tidal freshwater habitats.

SAMPLING LOCATIONS

- CUMBERLAND CHANNEL
- TIDAL CREEKS
- CUMBERLAND FLATS
- CUMBERLAND THOROUGHFARE

VALUES INDICATE DEPTH (MEAN LOW WATER) IN METERS

Figure 1-3b. View of Lilly Point marsh showing Big Creek (B), Holts Creek (H), Cumberland Thoroughfare (CT), and the adjacent Pamunkey River main-stem (P).


CT
B
H

Figure 1-4. Bongo-frame and plankton net assembly used to collect ichthyoplankton from the Pamunkey River.

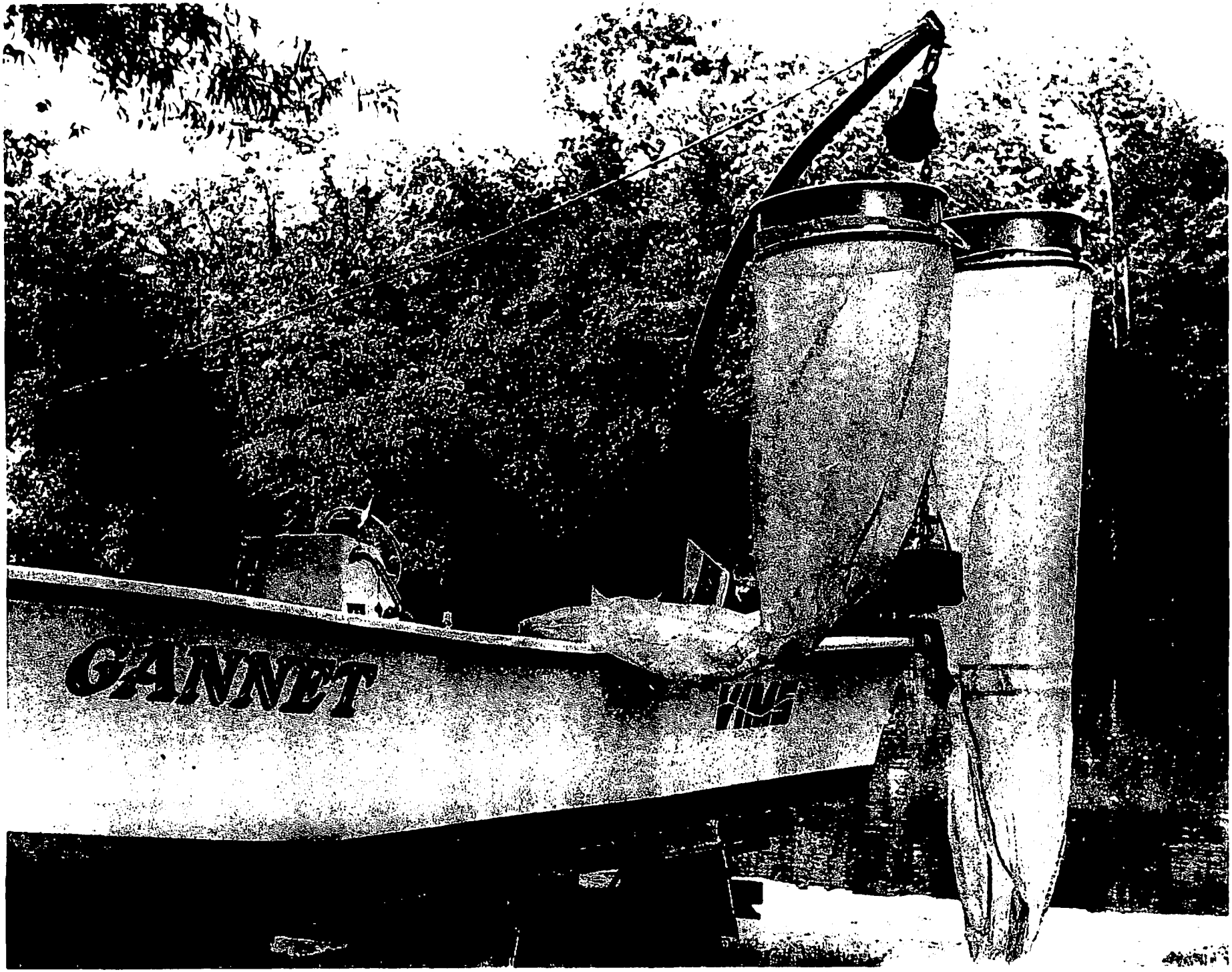

GANNET

Figure 1-5. Pushnet frame with paired plankton nets used to collect ichthyoplankton from potential tidal freshwater habitats of the Pamunkey River.

Figure 1-6. Dissolved oxygen concentration as a function of freshwater inflow in the tidal freshwater zone of the Pamunkey River for data collected during the 1989-1990 sampling seasons.

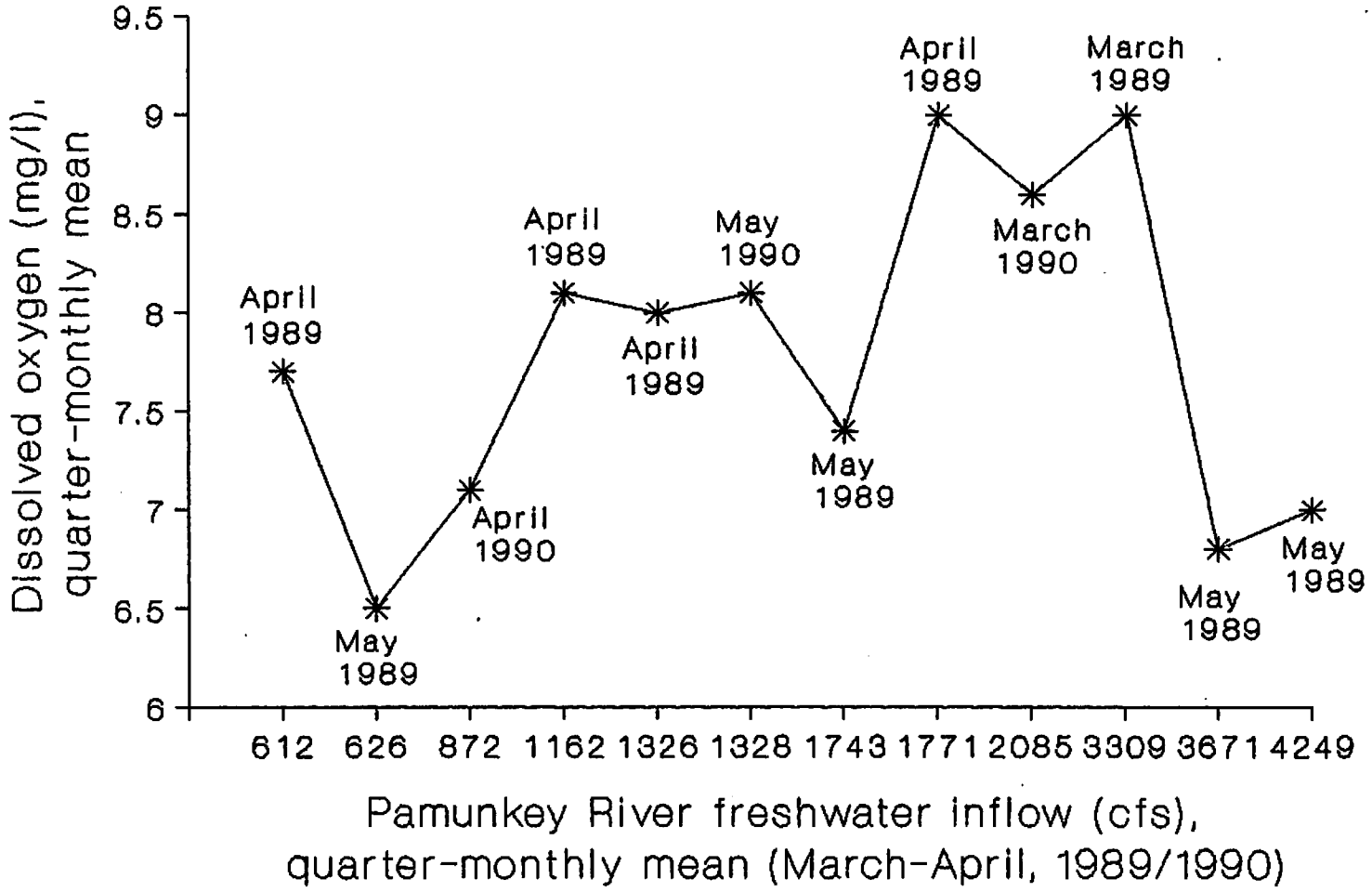

Dissolved oxygen (mg/l), quarter-monthly mean
9.5
9
8.5
8
7.5
7
6.5
6
April 1989
May 1989
April 1990
April 1989
April 1989
May 1990
May 1989
April 1989
March 1990
March 1989
May 1989
May 1989
612
626
872
1162
1326
1328
1743
1771
2085
3309
3671
4249
Pamunkey River freshwater inflow (cfs), quarter-monthly mean (March-April, 1989/1990)

Figure 1-7. Water transparency in tidal freshwater of the Pamunkey River during the 1989-1990 sampling seasons.

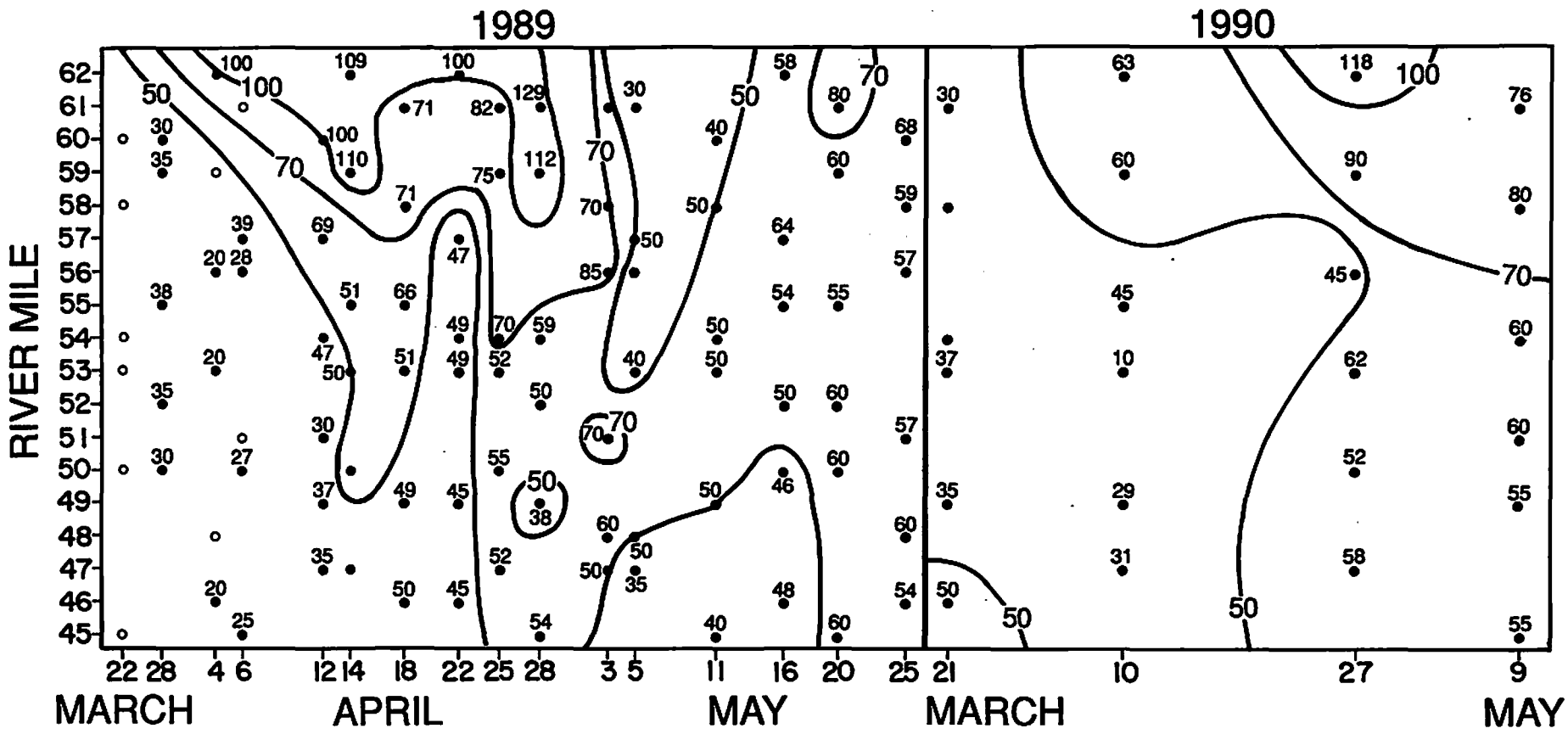
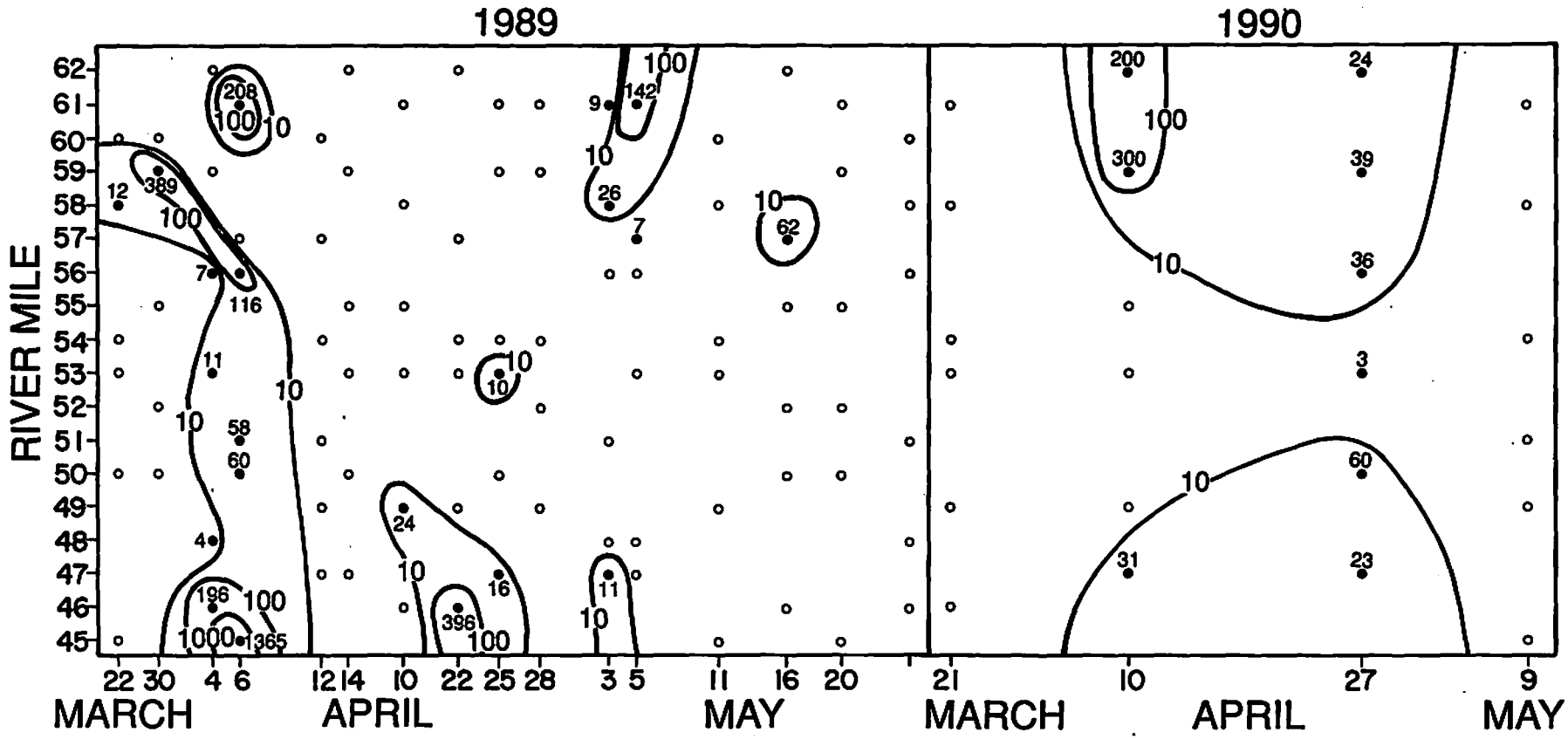

1989
1990
RIVER MILE
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
22 28 4 6 12 14 18 22 25 28 3 5 11 16 20 25 21 10 27 9
MARCH
APRIL
MAY
MARCH
MAY

Figure 1-8. Distribution of herring eggs in tidal freshwater of the Pamunkey River during the 1989-1990 sampling seasons.


1989
1990
RIVER MILE
62
61
60
59
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22 30 4 6 12 14 10 22 25 28 3 5 11 16 20 21 10 27 9
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MARCH
APRIL
MAY

Figure 1-9. Distribution of river herring prolarvae in tidal freshwater of the Pamunkey River during the 1989- 1990 sampling seasons.

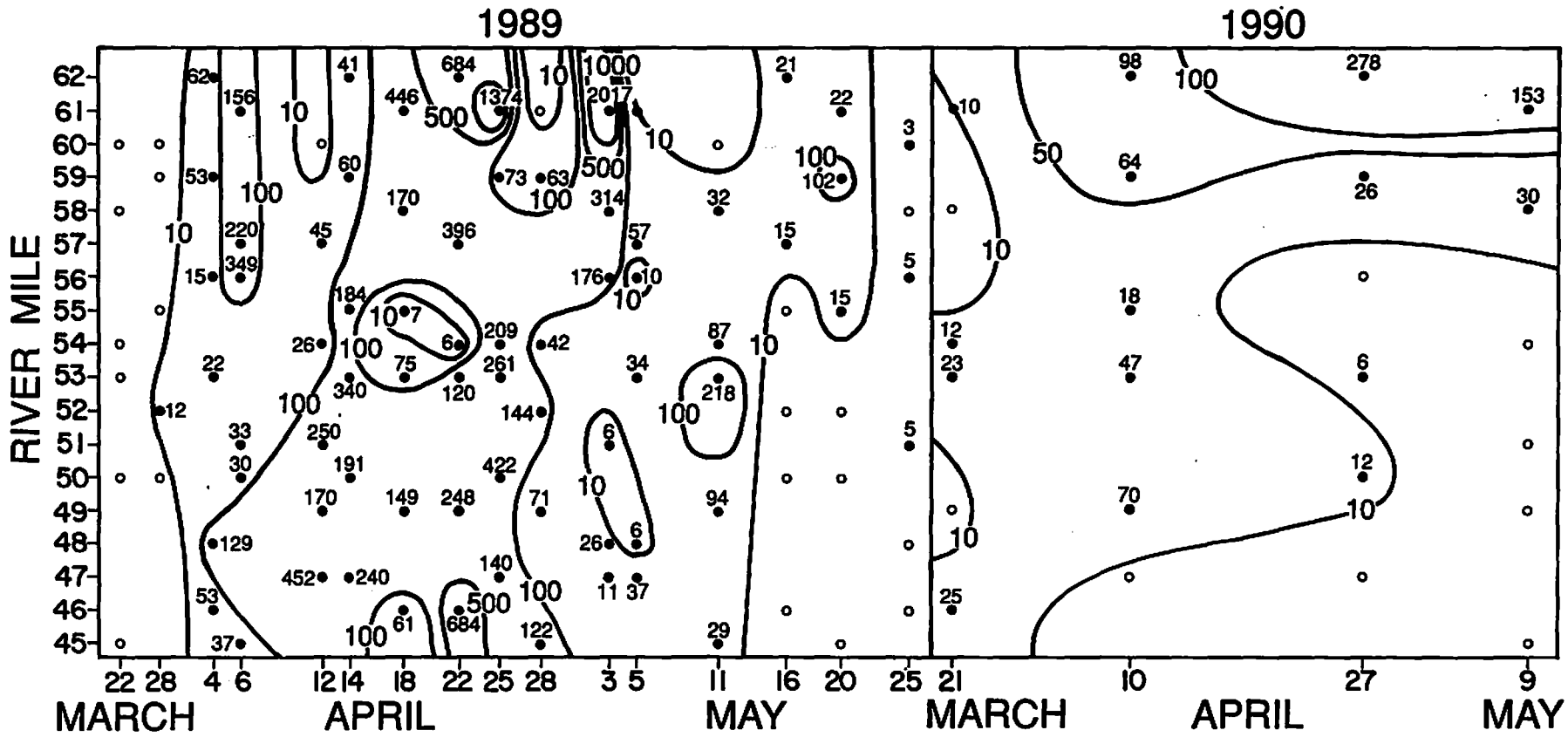
1989
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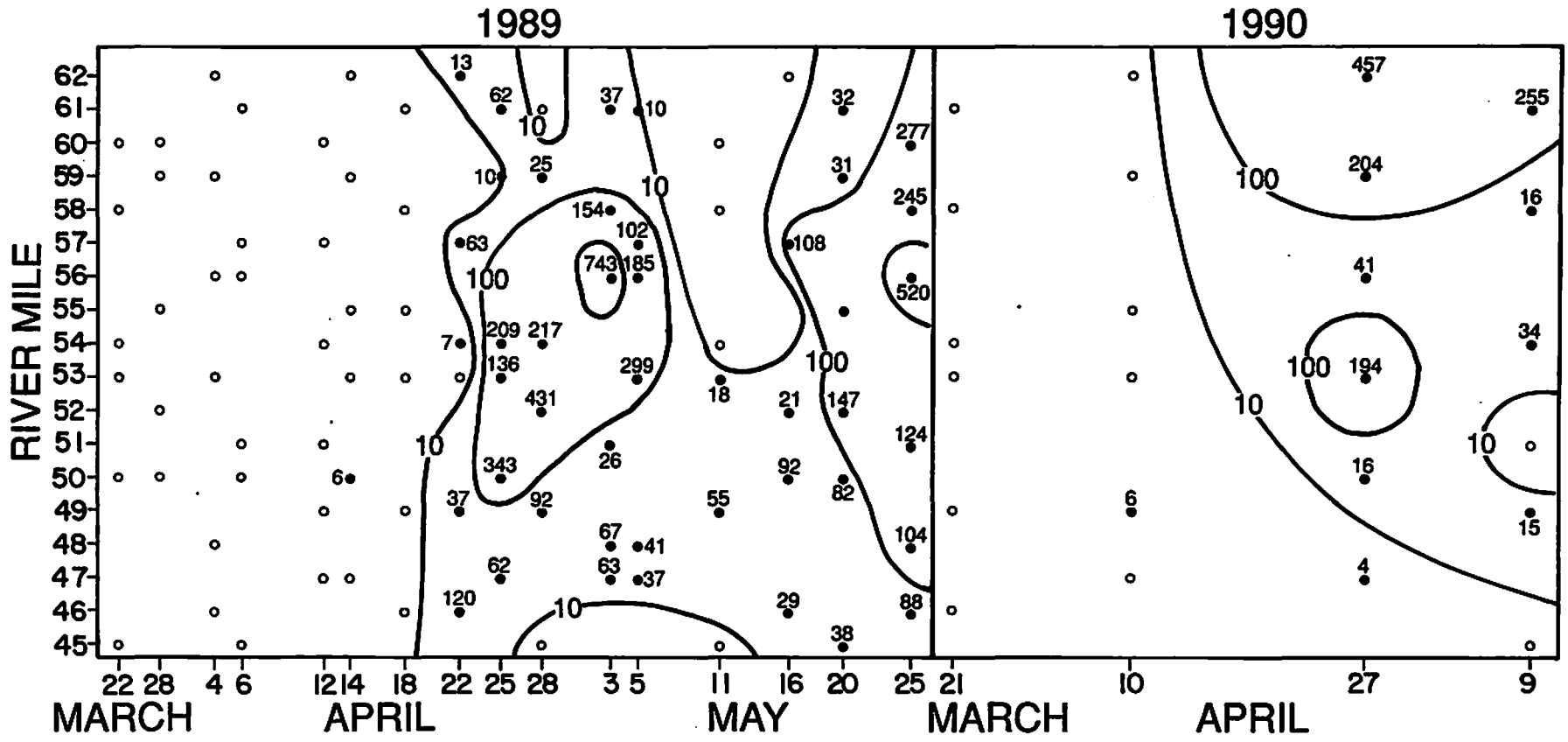
Figure 1-10. Distribution of gizzard shad prolarvae in tidal freshwater of the Pamunkey River during the 1989-1990 sampling seasons.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MARCH
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10
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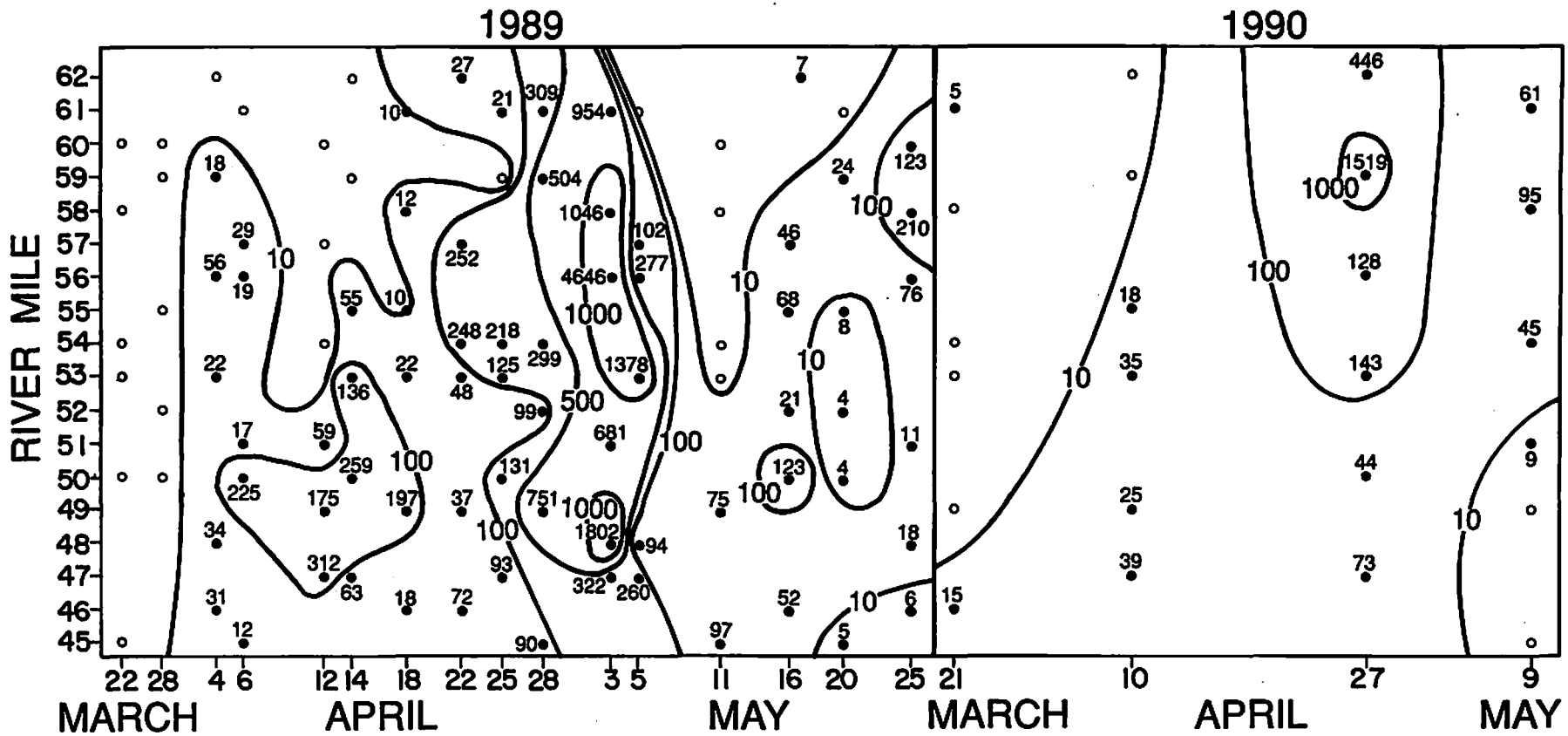
277
25
31
245
154
102
63
108
743
185
520
209
217
7
136
299
34
431
18
21
147
124
343
26
92
6
37
92
55
82
67
41
104
62
63
37
4
120
29
88
38
457
255
204
16
41
194
15
100
10

Figure 1-11. Distribution of herring postlarvae in tidal freshwater of the Pamunkey River during the 1989-1990 sampling seasons.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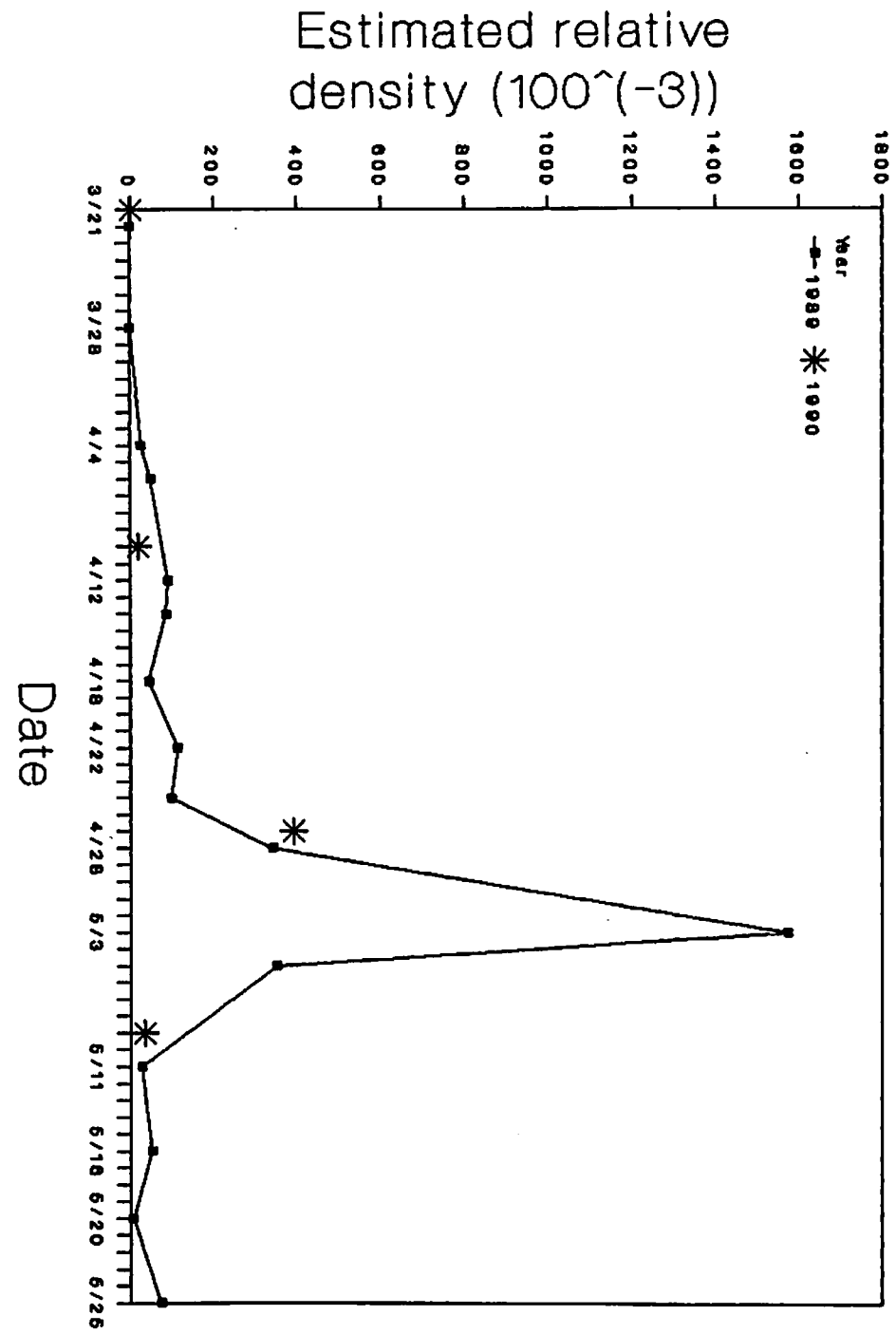
MARCH
APRIL
MAY
MARCH
APRIL
MAY

Figure 1-12. Estimated relative densities of river herring postlarvae in the Pamunkey River channel during the 1989-1990 sampling seasons.


Estimated relative density (100^(-3))
0
200
400
600
800
1000
1200
1400
1600
1800
Year
1989
1990
3/21
3/28
4/4
4/12
4/18
4/22
4/28
5/3
5/11
5/18
5/20
5/25
Date

PART II. Distributions of Potential Zooplankton Prey of River Herring in Tidal Freshwater of the Pamunkey River, Virginia.

SUMMARY

1. Collections of zooplankton were made to characterize the potential prey field of herring larvae in tidal freshwater of the Pamunkey River, Virginia. Samples were collected from the river main-stem and in potential tidal freshwater habitats at the same locations where ichthyoplankton were collected.

2. Five groups of zooplankton were enumerated, including *Keratella* spp. (a rotifer), "Rotifer spp.", copepod nauplii, copepodites, and cladocerans.

3. Relative densities of all zooplankton groups were higher downstream in the study area, and were typically higher in tidal creeks (Holts Creek and Big Creek) compared to the river main-stem and Cumberland Thoroughfare.

4. Relative densities of zooplankton were generally lower earlier during the sampling seasons and increased in late April-early May.

5. Rotifers exhibited the highest relative densities of the zooplankton groups. Relative densities of rotifers in the tidal creeks exceeded 1,000 L^{-1} in late April 1990 and early May 1989. Relative densities of rotifers were typically less than 100 L^{-1} in the study area along the river main-stem during both sampling seasons, although relative densities as high as about 600 L^{-1} were observed. Relative densities of nauplii were below 200 L^{-1}, and copepodites were below 100 L^{-1}. Relative densities of cladocerans were typically below 100 L^{-1}, although relative densities as high as about 500 L^{-1} were found.

6. Correlations between herring larval stages (prolarvae, preflexion larvae, and postflexion larvae) were generally weak to poor. Relatively higher coefficients of determination were observed between gizzard shad prolarvae and most zooplankton groups for collections made in potential tidal freshwater habitats. In contrast, coefficients of determination for correlations between river herring prolarvae and zooplankton were near zero, and most coefficients of correlation were negative. The difference is suggested to result from greater spawning by gizzard shad in tidal creeks later in the spawning season as zooplankton abundance increased.

7. Distributions of herring larvae and potential zooplankton prey appear to be independent or poorly correlated, possibly a consequence of patchy distributions of herring larvae and zooplankton within tidal freshwater.

PART II. Distributions of Potential Zooplankton Prey of River Herring in Tidal Freshwater of the Pamunkey River, Virginia.

Objective: Characterize the spatial and temporal distributions of potential zooplankton prey of herring larvae within the tidal freshwater zone of the Pamunkey River, Virginia.

INTRODUCTION

Coincident distribution of larval fish with suitable prey at high density has been hypothesized as one mechanism by which larvae avoid starvation (Hjort 1914; Cushing 1975; Lasker 1975, 1981). Experimental studies suggest that starvation may be significant only during the early larval stage for a relatively brief duration when stored energy reserves are at a minimum (Ehrlich 1974; Blaxter and Ehrlich 1974). As larvae develop they become less susceptible to starvation as they increase in mobility, consume larger prey, and increase the amount of energy stored as lipid and protein reserves (Ehrlich 1974).

Access to high concentrations of prey is important for the survival of fish larvae. Prey concentrations which exceed the average abundance appear necessary to insure sufficient encounter rates between fish larvae and their prey that lead to successfully completed acts of prey ingestion (Lasker and Zweifel 1977; Vlymen 1977; Hunter 1981). The availability of prey for river herring larvae within tidal freshwater may vary between locations within the river or between habitats. If so, then areas with greater concentrations of prey have potentially greater value as nursery habitat. The relative densities of zooplankton captured within tidal freshwater of the Pamunkey River, Virginia, are examined to characterize the spatial and temporal distributions of potential prey of herring larvae during the spring spawning season.

METHODS

Zooplankton were sampled at the same locations where ichthyoplankton were sampled (see Part I). To collect zooplankton, 20 L of river water were pumped from a standard depth of one meter using a 2000-GPH Rule pump powered by a 12-volt marine battery. Each sample was concentrated on a 50μ nylon-screen (NITEX) sieve, rinsed into a four-ounce sample jar, and fixed in 5% phosphate buffered formalin (5% PBF) (Markle 1984). An effort was made to minimize the volume of water used to wash the sieve to avoid excessive dilution of the preservative. If a jar was more than one-quarter full after the sieve was rinsed, the sample was reconcentrated and again rinsed into the jar. The final volume of most samples was at least three parts fixative to one part sample yielding a minimum estimated concentration of 3.75% formalin.

In the laboratory, the samples were adjusted to 110 mL with 5% PBF. Zooplankton in three one-mL aliquots from each sample were enumerated to estimate the relative densities of selected zooplankton groups. Subsamples were removed using a Stemple pipet and transferred to a Sedgewick-Rafter counting chamber. Zooplankton of potential importance as prey for herring larvae, including rotifers, nauplii and copepodites, and cladocerans, were enumerated using a binocular compound microscope at 40x magnification. Relative densities were estimated from the average of counts for the three aliquots. The distributions of larval herring stages and of potential zooplankton prey were analyzed using simple correlation to evaluate possible associations between herring larvae and zooplankton.

This is an initial analysis of the distribution of potential prey of herring larvae within the tidal freshwater nursery zone. It was not possible to complete the enumeration of zooplankton in all collected samples for presentation in this report due to the effort expended for

other components of this project. This work will continue as a component of doctoral dissertation research of the primary author to analyze the distribution and abundance of larval herring as a function of potential prey availability and abiotic factors that may influence larvae distribution.

RESULTS

The following results present an overview of the distributions of potential zooplankton prey in the tidal freshwater zone. Trends in the distribution of potential zooplankton prey of river herring larvae were observed both along the Pamunkey River channel and between potential tidal freshwater habitats. Enumeration of zooplankton groups in samples not included for this report will provide additional information on the relationships between herring larvae and their potential zooplankton prey in tidal freshwater.

Rotifera.

Two groups of rotifers, *Keratella* spp. and "Rotifera spp." (subsequently referred to as other rotifers), were enumerated in zooplankton collections. *Keratella* appears to have been the most common since relative densities of the two groups were typically similar; although, variation is evident. The second group was comprised of several taxa including *Notholca*, *Lepadella*, *Filinia*, *Lecane*, *Polyarthra*, *Trichocera*, *Asplanchna*, and bdelloid rotifers. Bdelloid rotifers and other soft-bodied rotifers, such as *Asplanchna*, were likely to have been underestimated since these are poorly fixed in formaldehyde without special treatment (Pennak 1989). The remaining groups, including *Keratella*, are loricate species which are more readily enumerated.

Tables 2-1 and 2-2 present estimated relative densities of *Keratella* and other rotifers, respectively, along the Pamunkey River channel and in Cumberland Thoroughfare during the two sampling seasons. Relative densities of both rotifer groups during both seasons were low in March and April; however, other rotifers appeared to increase earlier compared to *Keratella*. Higher relative densities of both groups in 1989 were observed on 3 May compared to remaining sampling dates for which data were available at the time of report preparation. During 3 May, the relative density of other rotifers exceeded the relative density of *Keratella*, especially in the downstream strata of the study area. High river flow, predation, or a combination of these or other factors appear to have caused a marked decline in the relative density of other rotifers by 5 May. On 5 May, relative densities of both rotifer groups were nearly identical. Relative densities of *Keratella* and other rotifers in Cumberland Thoroughfare were similar to relative densities in strata downstream in the study area. Relative densities of *Keratella* and other rotifers appeared to increase in the downstream half of the study area compared to the upstream half; however, relative densities in stratum 4 were higher in 1990 compared to 1989 suggesting that the zone of higher productivity might have encroached upstream due to lower runoff (see Part I). Mean relative densities of these groups in the area of lower apparent zooplankton abundance as well as in Cumberland Thoroughfare and during March and April were similar between the two sampling seasons.

Tables 2-3 and 2-4 present estimated relative densities of *Keratella* and other rotifers, respectively, in the potential tidal freshwater habitats. Relative densities of both groups were substantially higher in the two tidal creeks during 1990 compared to 1989. Differences in the relative densities of other rotifers in

collections from Holts Creek earlier in the day and later in the day do not appear to be significant and may represent variability of the system. In contrast, *Keratella* appears to exhibit much higher variability between diel samples with highest mean relative density . occurring earlier in the day. The relatively smaller difference between diel relative densities of other rotifers may result from masking diel distributions of individual taxa within the larger assemblage. In both seasons, relative densities of other rotifers appeared to be higher over the flats and in Cumberland Thoroughfare compared to over the channel. This trend was observed for *Keratella* in 1990 but not in 1989.

Copepoda.

Table 2-5 presents estimated relative densities of copepod nauplii in strata along the river main-stem and in Cumberland Thoroughfare, and Table 2-6 presents estimated relative densities for this group in the potential tidal freshwater habitats. Mean relative densities of copepod nauplii within the river main-stem appear to have been higher in March, 1989, compared to March, 1990, but were of the same magnitude in early April of both seasons. In comparison, relative density of nauplii peaked in late April 1990, but no increase was observed during this time period in 1989. Differences during March and April of the two sampling seasons may be related to river flow which was higher in March than in April, 1989, but was higher in April than in March, 1990 (Part I). Higher river flow may have influence the development of the spring phytoplankton bloom on which nauplii may depend for food, although other factors such as water temperature may also have influenced these results. Relative density was generally higher downstream in the study area. Mean relative densities of nauplii were generally higher in Cumberland Thoroughfare than in the river main-stem earlier in the

season, but were lower in the thoroughfare later in the season. Relative densities of nauplii often were higher in tidal creeks compared to the remaining potential habitats. Relative densities of copepodites were comparable between samples collected in the Pamunkey River main-stem and in Cumberland Thoroughfare (Table 2-7) and between potential tidal freshwater habitats (Table 2-8). Results suggest that copepodites exhibit higher relative density in tidal creeks compared to the main-stem channel; however, these results must be interpreted with caution since gear avoidance by copepodites appears to have been substantial, especially in the river main-stem.

Cladocera.

Relative densities of cladocerans in the Pamunkey River main-stem (Table 2-9) were low in 1989 from March to mid-April, but increased markedly by late April-early May, 1989. A similar increase from early to late April, 1990, was not observed. As noted for Rotifera and copepod nauplii, the relative density of cladocerans was typically higher downstream in the study area. The relative density of cladocerans in Cumberland Thoroughfare was generally higher compared to the river main-stem in 1989, but was generally lower compared to the river main-stem during the 1990 season. Relative densities of cladocerans in the potential tidal freshwater habitats prior to 30 May 1989 and in 1990 were highest in Holts Creek and were of similar magnitude in the remaining sites (Table 2-10). In comparison, the relative density of cladocerans was higher in Big Creek compared to Holts Creek on 30 May 1989.

Total zooplankton potential prey.

Relative density of potential prey (sum of all potential prey groups) in the river main-stem was similar in both seasons; relative density was low from early to mid-April, subsequently increased during late April, and was highest in May (Table 2-11). The relative density of potential prey in the downstream portion of the study area was greater in March, 1989, than in March, 1990. Relative densities were generally lower in strata 5 and 6 than in strata further downstream or in Cumberland Thoroughfare. Relative densities of potential prey was substantially higher in tidal creeks compared to the remaining sampling sites, and was frequently higher in Holts Creek compared to Big Creek (Table 2-12).

Associations between herring larvae and potential prey.

Table 2-13 summarizes the associations between herring larvae and zooplankton captured in the main-stem Pamunkey River. Correlation coefficients ranged from poor (near zero) to weak, and most were non-significant. Significant correlations were found between preflexion larvae and Rotifera spp., Cladocera, and total zooplankton. Correlations of river herring prolarvae and gizzard shad prolarvae with potential prey groups were non-significant. Most coefficients for associations with gizzard shad prolarvae were near zero, but most coefficients for associations with river herring were negative suggesting that spawning by river herring occurred primarily outside of the zones of higher zooplankton abundance.

Table 2-14 summarizes the associations between herring larvae and zooplankton captured in potential tidal freshwater habitats within the Pamunkey River. Correlation coefficients ranged from low to moderate for postlarvae, were highest for gizzard shad prolarvae, and were

generally lower for river herring prolarvae. Correlations between river herring prolarvae and zooplankton were non-significant, and most correlation coefficients were negative. In contrast, significant correlations were found between gizzard shad prolarvae and rotifers, nauplii, and total zooplankton. Preflexion larvae also exhibited significant correlations with rotifers, nauplii, and total zooplankton, but postflexion larvae were significantly correlated only with rotifers and total zooplankton. Similar associations were found between preflexion larvae and Rotifera spp. and total zooplankton for collections made both along the mainstem channel and between potential habitats.

Observed correlations are suggested to arise from distribution patterns of herring larvae and zooplankton rather than due to cause-effect relationships arising from trophic interactions. Negative correlations between river herring prolarvae and zooplankton are suggested to arise from the occurrence of this group in the study area earlier in the season during lower zooplankton abundance. Also, higher relative densities of this group occurred in the upstream reach of the study area where zooplankton densities were usually lower. Relative densities of gizzard shad prolarvae and herring postlarvae, on the other hand, were higher in the downstream reach of the study area where zooplankton concentrations were higher. Also, gizzard shad typically begin to spawn in late April at about the time when zooplankton densities appear to begin to increase. The results suggest that downstream advection of river herring larvae into the zone of higher productivity may be important for growth and survival, but larval transport may not be as important for gizzard shad since this species appears to spawn in this zone.

DISCUSSION

Elevated concentrations of zooplankton observed downstream in the study area are suggested to be associated with the upper, low-salinity zone of the York-Pamunkey estuary. Van Engel and Joseph (1968) defined the approximate upper limit of this estuary at P50, although tide stage and river flow cause variation in the absolute position of this zone. Van Engel and Joseph reported mean salinity (12-yr average, 1955-1966) of 0.2 ppt (range: 0.0-1.8 ppt) at this level with lowest salinities occurring in late winter and early spring, and they found highest dissolved oxygen concentration, 100% saturation throughout the year, at this level compared to the remainder of the estuary, except at the mouth of the York River and in Chesapeake Bay. The lower third of the study area for this investigation (P45 to P50) occurred within the upper estuarine zone as defined by Van Engel and Joseph (1968).

The upper estuary is an area of great biological and chemical significance to the estuarine ecosystem (Morris et al. 1978). Elevated concentrations of rotifers, copepod nauplii, and cladocerans in this zone are suggested to result from higher reproduction rates supported by high concentrations of diatoms and detritus that occur in this zone (Van Engel and Joseph 1968; Anderson 1986; Marshall and Alden 1990). Higher nutrient concentrations are found in this upper estuary zone compared to areas farther upstream or downstream (Marshall and Alden 1990). Higher concentrations of dissolved reactive silica and ammonia and concentrations of dissolved phosphorous occur in the upper estuary from late autumn to early spring prior to the spring phytoplankton bloom (Anderson 1986; Marshall and Alden 1990). Transport of nutrients, plankton, and detritus from upstream areas and export from adjacent marshes may also contribute to higher concentrations in the upper estuary (Odum 1984; Odum et al. 1984). Contributions of these

components may vary depending on their source, river flow, tidal flushing and other factors. Heinle and Flemer (1976) found little export of organic carbon from a poorly flooded tidal marsh, but found export of nitrogen and phosphorous, primarily in dissolved forms.

In the Pamunkey River, Van Engel and Joseph (1968) reported high concentration of detritus in the reach from P30 to P50. Bacteria associated with detritus has been suggested to be an important energy source for zooplankton in the upper estuary zone (Heinle et al. 1973; Odum et al. 1984; Sellner 1988). Bacteria are an important component of the tidal freshwater-upper estuarine zone, and play a primary role in the regeneration of nutrients by metabolizing labile organic nutrients in detritus and possibly metabolizing dissolved organic carbon (Morris et al. 1978; Odum et al. 1984). Morris et al. (1978) demonstrated that the dynamics of dissolved oxygen in the low salinity zone of the Tamar estuary could only be explained when metabolism by the bacterial population was accounted for in their analysis. Fenchel (1972 cited in Heinle et al. 1974) hypothesized that a detrital food web increases stability in ecosystems by making energy fixed by primary producers during the growing season available to higher order consumers over a longer period. However, considerable debate remains regarding the role of bacteria as "link or sink" between detrital biomass and higher order consumers (Sherr et al. 1987).

Rotifers, cladocerans, and copepods (primarily nauplii) are the primary potential prey of river herring larvae (Norden 1968; Nigro and Ney 1982; Crecco and Blake 1983). Most rotifers are omnivorous and consume all organic particles of appropriate size, and cladocerans consume all organic particles of appropriate size with organic detritus and bacteria comprising the bulk of ingested material (Pennak 1989). Also, detritus appears to be a significant component of the diet of

copepods in estuaries and tidal marshes (Heinle et al. 1974; Heinle and Flemer 1976; Pennak 1989). Consequently, dynamics of a detrital food web in the tidal freshwater-upper estuary nursery zone may be significant to the survival and growth of herring larvae, especially during the early spring prior to development of the diatom bloom.

In the vicinity of the fresh-salt interface, tidal creeks appear to be characterized by high levels of secondary productivity; relative densities of rotifers, nauplii, and cladocerans were typically higher in both Holts Creek and Big Creek compared to the river main-stem or the thoroughfare. High productivity of zooplankton in tidal creeks at this level is likely to be associated with nutrient dynamics of the adjacent tidal wetland, and may be influence by the relative importance of phytoplankton and detritus as energy and nutrient sources for higher consumers (Wylie and Currie 1991). Other mechanisms by which significant quantities of nutrients might be imported into the tidal freshwater marsh system include decomposition of carcasses (Durbin et al. 1979; Threlkeld 1988; Parmenter and Lamarra, 1991), excretion (Brabrand et al. 1990), or in fecal pellets which enter the detritus (Rothans and Miller 1991).

Differences between the potential habitats surveyed for this study indicate significant heterogeneity within the tidal freshwater-upper estuary zone. Elevated concentrations of herring larvae in Holts Creek compared to Big Creek and the remaining habitats suggest that herring larvae respond to heterogeneity in their environment, although predation can not be excluded as a determinant of herring larvae distributions (see Part I).

The tidal freshwater ecosystem is poorly understood. Further research is necessary to elaborate temporal and spatial distributions of potential zooplankton prey within this ecosystem that may influence

inter- and intra-annual variation in the quality of the nursery habitat, and the relationship between potential prey and larval fish as it relates to the establishment of year class strength.

LITERATURE CITED

Anderson, G. F. 1986. Silica, diatoms and a freshwater productivity maximum in Atlantic coastal plain estuaries, Chesapeake Bay. Estuarine, Coastal and Shelf Science 22: 183-197.

Blaxter, J. H. S. and K. F. Ehrlich. 1974. Changes in behavior during starvation of herring and plaice larvae, pages 575-588. *In*: J. H. S. Blaxter (editor), The early life history of fish. Springer-Verlag, Berlin.

Brabrand, A., B. A. Faafeng, and J. P. M. Nilssen. 1990. Relative importance of phosphorus supply to phytoplankton production: fish excretion versus external loading. Canadian Journal of Fisheries and Aquatic Sciences 47: 364-372.

Crecco, V. and M. M. Blake. 1983. Feeding ecology of coexisting larvae of American shad and blueback herring in the Connecticut River. Transactions of the American Fisheries Society 112: 498-507.

Cushing, D. H. 1975. Marine ecology and fisheries. Cambridge University Press. 278 p.

Durbin, A. G., S. W. Nixon and C. A. Oviatt. 1979. Effects of the spawning migration of the alewife, *Alosa pseudoharengus*, on freshwater ecosystems. Ecology 60: 8-17.

Ehrlich, K. F. 1974. Chemical changes during growth and starvation of herring larvae, pages 301-324. *In*: J. H. S. Blaxter (editor), The early life history of fish. Springer-Verlag, Berlin.

Fenchel, T. 1972. Aspects of decomposer food chains in marine benthos. Verh. d. Dtsch. Zool. Ges. 65: 14-23. (not seen).

Heinle, D. R., D. A. Flemer. 1976. Flows of materials between poorly flooded tidal marshes and an estuary. Marine Biology 35: 359-373.

Heinle, D. R., D. A. Flemer, J. F. Ustach, R. A. Murtagh, and R. P. Harris. 1974. The role of organic debris and associated microorganisms in pelagic estuarine food chains. University of Maryland Water Resources Research Center Technical Report 22. 54 pp.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapports et Proces-Verbaux des Reunions, Consel International pur l'Exploration de la Mer 20: 1-228.

Hunter, J. R. 1981. Feeding ecology and predation of marine fish larvae, pages 33-77. *In*: R. Lasker (editor), Marine fish larvae. University of Washington Sea Grant, Seattle. 131 p.

Lasker, R. 1975. Field criteria for survival of anchovy larvae: the relation between inshore chlorophyll maximum layers and successful first feeding. Fishery Bulletin, U.S. 73: 453-462

Lasker, R. 1981. The role of a stable ocean in larval fish survival and subsequent recruitment, pages 80-87. *In*: R. Lasker (editor),

Marine fish larvae. University of Washington Sea Grant, Seattle. 131 p.

Lasker, R. and J. R. Zweifel. 1977. Growth and survival of first-feeding northern anchovy (*Engraulis mordax*) in patches containing different proportions of large and small prey, pages 329-354. *In*: J.H. Steele (editor), Spatial pattern in plankton communities. Plenum Press, New York. 470 p.

Markle, D. F. 1984. Phosphate buffered formalin for long term preservation of formalin fixed ichthyoplankton. Copeia 1984: 525-528.

Marshall, H. G. and R. W. Alden. 1990. A comparison of phytoplankton assemblages and environmental relationships in three estuarine river of the lower Chesapeake Bay. Estuaries 13: 287-300.

Morris, A. W., R. F. C. Mantoura, A. J. Bale, and R. J. M. Howland. 1978. Very low salinity regions of estuaries: important sites for chemical and biological reactions. Nature 274: 678-680.

Nigro, A. A. and J. J. Ney. 1982. Reproduction and early life history accommodations of landlocked alewives to a southern range extension. Transactions of the American Fisheries Society 111: 559-569.

Norden, C. R. 1968. Morphology and food habits of the larval alewife, *Alosa pseudoharengus* (Wilson), in Lake Michigan. Journal of Great Lakes Research 11: 103-110.

Odum, W. E. 1984. Dual-gradient concept of detritus transport and processing in estuaries. Bulletin of Marine Science 35: 510-521.

Odum, W. E., T. J. Smith III, J. K. Hoover, and C. C. McIvor. 1984. The ecology of tidal freshwater marshes of the United States east coast: a community profile. U.S. Fish and Wildlife Service, FWS/OBS-83/17. 177 pp.

Parmenter, R. R. and V. A. Lamarra. 1991. Nutrient cycling in a freshw ater marsh: the decomposition of fish and waterfowl carrion. Limnology and Oceanography 36: 976-987.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States, 3rd edition: Protozoa to mollusca. J. Wiley and Sons, New York. 628 pp.

Rothans, T. C. and A. C. Miller. 1991. A link between biologically imported particulate organic nutrients and the detritus food web in reef communities. Marine Biology 110: 145-150.

Sellner, K. G. 1988. The importance of the microbial loop in the Chesapeake Bay and its tributaries, pages 60-64. *In*: M. P. Lynch and E. C. Krome (editors), Understanding the estuary: advances in Chesapeake Bay research. Chesapeake Research Consortium Publication Number 129, CBP/TRS 24/88.

Sherr, E. B., B. F. Sherr, and L. J. Albright. 1987. Bacteria: link or sink? Science 235: 88-89.

Threlkeld, S. T. 1988. Planktivory and planktivore biomass effects on zooplankton, phytoplankton, and the trophic cascade. Limnology and Oceanography 33: 1362-1375.

Van Engel, W. A. and E. B. Joseph. 1968. Characterization of coastal and estuarine fish nursery grounds as natural communities. Final Report, Bureau of Commercial Fisheries, Virginia Institute of Marine Science. 43 p.

Vlymen, W. J. 1977. A mathematical model of the relationship between larval anchovy (*Engraulis mordax*) growth, prey microdistribution, and larval behavior. Environmental Biology of Fish 2: 211-233.

Wylie, J. L. and D. J. Currie. 1991. The relative importance of bacteria and algae as food sources for crustacean zooplankton. Limnology and Oceanography 36: 708-728.

Table 2-1. Relative density (L^{-1}) of potential zooplankton prey in tidal freshwater of the Pamunkey River, Virginia, and in Cumberland Thoroughfare: *Keratella* spp. (Dash indicates missing collection.)

Location	Date						Mean
	1989						
	March	April			May		
	22	12	14	28	3	5	
P45-47 (1)	-	9	0	30	83	109	46
P48-50 (2)	7	3	0	5	164	94	45
P51-53 (3)	6	0	0	39	178	33	43
P54-56 (4)	-	4	2	15	42	6	14
P57-59 (5)	-	0	0	0	3	11	3
P60-62 (6)	3	7	6	0	4	0	4
Thoroughfare	-	0	-	9	158	124	58
Mean	5	3	2	14	90	54	
	1990						
	March	April			May		
	21	10		27	9		
P45-47 (1)	3	12		12	444		118
P48-50 (2)	6	3		18	246		68
P51-53 (3)	0	6		3	306		79
P54-56 (4)	3	0		9	276		72
P57-59 (5)	3	6		0	3		3
P60-62 (6)	8	0		0	0		2
Thoroughfare	6	9		24	394		108
Mean	4	5		10	238		

Table 2-2. Relative density (L^{-1}) of potential zooplankton prey in tidal freshwater of the Pamunkey River, Virginia, and in Cumberland Thoroughfare: Rotifera spp. (Dash indicates missing collection.)

Location	Date						Mean
	1989						
	March	April			May		
	22	12	14	28	3	5	
P45-47 (1)	-	0	15	103	192	179	98
P48-50 (2)	18	4	6	127	846	106	185
P51-53 (3)	11	9	9	52	585	32	116
P54-56 (4)	-	0	6	73	47	9	26
P57-59 (5)	-	0	0	0	26	3	6
P60-62 (6)	5	18	9	0	12	39	12
Thoroughfare	-	18	-	18	218	36	73
Mean	11	7	6	53	275	58	
	1990						
	March	April			May		
	21	10		27	9		
P45-47 (1)	60	21		73	217		93
P48-50 (2)	6	9		91	64		42
P51-53 (3)	6	3		18	100		32
P54-56 (4)	0	0		33	79		28
P57-59 (5)	0	6		6	14		7
P60-62 (6)	9	15		0	0		6
Thoroughfare	6	39		121	152		80
Mean	13	14		49	89		

Table 2-3. Relative density (L^{-1}) of potential zooplankton prey in potential tidal freshwater habitats of the Pamunkey River, Virginia: *Keratella* spp. (Dash indicates missing collection.)

Location	Date					Mean
	1989					
	April		May			
	20		18	30		
Holts Creek, early	7		90	7873		2657
Holts Creek, late	-		-	379		
Big Creek	21		15	1939		658
Channel	8		2	244		85
Flats	3		13	676		231
Thoroughfare	5		17	218		80
Mean	3		27	1888		
	1990					
	April			May		
	16	22	29	8	12	
Holts Creek, early	3	152	5573	4299	4943	2994
Holts Creek, late	5	922	318	4823	-	907
Big Creek	8	59	115	4823	4294	1860
Channel	2	3	38	26	64	26
Flats	0	6	64	117	292	96
Thoroughfare	0	5	52	292	530	176
Mean	3	191	1027	1990	2025	

Table 2-4. Relative density (L^{-1}) of potential zooplankton prey in potential tidal freshwater habitats of the Pamunkey River, Virginia: Rotifera spp. (Dash indicates missing collection.)

Location	Date					Mean
	1989					
	April		May			
	20		18	30		
Holts Creek, early	14		347	5405		1922
Holts Creek, late	-		-	8612		
Big Creek	27		75	1518		540
Channel	6		10	192		69
Flats	3		45	930		326
Thoroughfare	7		47	374		143
Mean	11		105	2839		
	1990					
	April			May		
	16	22	29	8	12	
Holts Creek, early	182	387	7223	3909	2726	2885
Holts Creek, late	23	1557	496	5274	-	1837
Big Creek	196	554	155	2232	932	815
Channel	5	8	23	9	11	11
Flats	8	6	67	41	105	45
Thoroughfare	6	46	108	56	100	63
Mean	70	427	1345	1920	775	

Table 2-5. Relative density (L^{-1}) of potential zooplankton prey in tidal freshwater of the Pamunkey River, Virginia, and in Cumberland Thoroughfare: nauplii. (Dash indicates missing collection.)

Location	Date						Mean
	1989						
	March	April			May		
	22	12	14	28	3	5	
P45-47 (1)	-	24	24	9	11	30	20
P48-50 (2)	108	43	24	36	115	12	56
P51-53 (3)	56	15	36	18	112	23	44
P54-56 (4)	-	12	9	32	20	9	16
P57-59 (5)	-	12	6	6	20	10	11
P60-62 (6)	17	9	0	24	0	12	12
Thoroughfare	-	24	-	21	21	9	19
Mean	60	20	17	21	44	15	
	1990						
	March	April			May		
	21	10		27	9		
P45-47 (1)	21	12		109	96		60
P48-50 (2)	29	8		64	58		39
P51-53 (3)	12	30		183	82		77
P54-56 (4)	6	3		167	109		71
P57-59 (5)	0	5		336	6		87
P60-62 (6)	8	9		24	3		11
Thoroughfare	12	21		94	49		44
Mean	13	13		140	57		

Table 2-6. Relative density (L^{-1}) of potential zooplankton prey in potential tidal freshwater habitats of the Pamunkey River, Virginia: nauplii. (Dash indicates missing collection.)

Location	Date					Mean
	1989					
	April		May			
	20		18	30		
Holts Creek, early	109		113	226		149
Holts Creek, late	-		-	152		
Big Creek	77		54	332		154
Channel	21		12	80		38
Flats	17		42	120		60
Thoroughfare	18		17	199		78
Mean	48		48	185		
	1990					
	April			May		
	16	22	29	8	12	
Holts Creek, early	21	66	192	138	188	121
Holts Creek, late	49	167	224	108	-	137
Big Creek	65	100	226	138	85	123
Channel	49	58	188	36	26	71
Flats	44	26	173	55	47	69
Thoroughfare	39	44	115	63	56	63
Mean	44	77	186	89	80	

Table 2-7. Relative density (L^{-1}) of potential zooplankton prey in tidal freshwater of the Pamunkey River, Virginia, and in Cumberland Thoroughfare: copepodites. (Dash indicates missing collection.)

Location	Date						Mean
	1989						
	March	April			May		
	22	12	14	28	3	5	
P45-47 (1)	-	6	3	3	3	3	4
P48-50 (2)	9	1	3	0	0	3	3
P51-53 (3)	11	9	27	0	6	6	10
P54-56 (4)	-	5	6	5	0	0	3
P57-59 (5)	-	6	0	0	3	0	2
P60-62 (6)	2	11	0	0	4	6	4
Thoroughfare	-	3	-	18	9	12	11
Mean	7	6	7	4	4	4	
	1990						
	March	April			May		
	21	10		27	9		
P45-47 (1)	0	3		0	2		1
P48-50 (2)	0	2		3	15		5
P51-53 (3)	0	0		17	0		4
P54-56 (4)	0	0		30	3		8
P57-59 (5)	0	0		0	0		0
P60-62 (6)	2	0		3	0		1
Thoroughfare	0	0		6	15		5
Mean	<1	<1		8	5		

Table 2-8. Relative density (L^{-1}) of potential zooplankton prey in potential tidal freshwater habitats of the Pamunkey River, Virginia: copepodites. (Dash indicates missing collection.)

Location	Date					Mean
	1989					
	April		May			
	20		18	30		
Holts Creek, early	68		10	58		45
Holts Creek, late	-		-	55		
Big Creek	31		2	139		58
Channel	0		1	14		5
Flats	0		2	96		33
Thoroughfare	5		1	56		21
Mean	21		3	70		
	1990					
	April			May		
	16	22	29	8	12	
Holts Creek, early	0	23	20	5	11	12
Holts Creek, late	0	13	21	12	-	12
Big Creek	5	2	26	3	11	9
Channel	0	5	39	9	2	11
Flats	2	0	9	8	9	6
Thoroughfare	0	7	17	11	15	10
Mean	1	8	22	8	9	

Table 2-9. Relative density (L^{-1}) of potential zooplankton prey in tidal freshwater of the Pamunkey River, Virginia, and in Cumberland Thoroughfare: Cladocera. (Dash indicates missing collection.)

Location	Date						Mean
	1989						
	March	April			May		
	22	12	14	28	3	5	
P45-47 (1)	-	0	0	609	192	107	182
P48-50 (2)	0	2	0	120	821	30	162
P51-53 (3)	8	0	3	27	245	12	51
P54-56 (4)	-	0	0	229	35	12	55
P57-59 (5)	-	0	3	3	27	0	7
P60-62 (6)	0	2	0	0	4	0	1
Thoroughfare	-	6	-	149	161	0	79
Mean	3	1	1	162	214	23	
	1990						
	March	April			May		
	21	10		27	9		
P45-47 (1)	15	0		42	2		15
P48-50 (2)	3	3		24	0		8
P51-53 (3)	3	15		21	3		11
P54-56 (4)	6	0		0	0		2
P57-59 (5)	3	0		3	2		2
P60-62 (6)	0	3		5	3		3
Thoroughfare	0	0		24	0		6
Mean	4	3		17	1		

Table 2-10. Relative density (L^{-1}) of potential zooplankton prey in potential tidal freshwater habitats of the Pamunkey River, Virginia: Cladocera. (Dash indicates missing collection.)

Location	Date					Mean
	1989					
	April		May			
	20		18	30		
Holts Creek, early	59		81	235		125
Holts Creek, late	-		-	67		
Big Creek	27		3	358		129
Channel	8		3	70		27
Flats	16		3	153		57
Thoroughfare	18		11	77		36
Mean	26		21	160		
	1990					
	April			May		
	16	22	29	8	12	
Holts Creek, early	3	13	76	47	23	32
Holts Creek, late	3	11	15	12	-	10
Big Creek	0	11	11	5	9	7
Channel	0	6	11	0	0	3
Flats	3	3	5	2	8	4
Thoroughfare	11	7	21	8	0	9
Mean	3	9	23	12	8	

Table 2-11. Relative density (L^{-1}) of potential zooplankton prey in tidal freshwater of the Pamunkey River, Virginia, and in Cumberland Thoroughfare: total zooplankton. (Dash indicates missing collection.)

Location	Date						Mean
	1989						
	March	April			May		
	22	12	14	28	3	5	
P45-47 (1)	-	39	42	755	482	427	349
P48-50 (2)	142	52	33	288	1946	246	451
P51-53 (3)	91	33	76	136	1136	106	263
P54-56 (4)	-	21	21	353	143	115	115
P57-59 (5)	-	18	9	9	79	28	28
P60-62 (6)	16	48	9	24	31	31	31
Thoroughfare	-	52	-	215	567	254	254
Mean	83	38	32	254	626	154	
	1990						
	March	April			May		
	21	10		27	9		
P45-47 (1)	100	49		236	759		286
P48-50 (2)	44	24		200	382		163
P51-53 (3)	21	55		242	491		202
P54-56 (4)	15	3		239	467		181
P57-59 (5)	6	17		346	24		98
P60-62 (6)	26	27		32	6		23
Thoroughfare	24	70		270	609		243
Mean	34	35		224	391		

Table 2-12. Relative density (L^{-1}) of potential zooplankton prey in potential tidal freshwater habitats of the Pamunkey River, Virginia: total zooplankton. (Dash indicates missing collection.)

Location	Date					Mean
	1989					
	April		May			
	20		18	30		
Holts Creek, early	257		642	13796		4898
Holts Creek, late	-		-	9264		
Big Creek	183		150	4286		1499
Channel	43		29	600		249
Flats	41		105	1974		703
Thoroughfare	52		94	924		488
Mean	115		204	5141		
	1990					
	April			May		
	16	22	29	8	12	
Holts Creek, early	209	641	13083	8397	7890	6044
Holts Creek, late	79	2670	1074	7790	-	2903
Big Creek	273	730	532	7200	5330	2813
Channel	55	80	299	80	102	123
Flats	56	41	317	221	461	219
Thoroughfare	53	108	312	429	702	321
Mean	121	712	2603	4020	2897	

Table 2-13. Correlations between herring larvae and potential zooplankton prey captured in the Pamunkey River from P45 to P62 and in Cumberland Thoroughfare (* $\alpha<0.05$, ** $\alpha<0.01$).

Potential Zooplankton Prey	River herring prolarvae	Gizzard shad prolarvae	Preflexion larvae
Keratella spp.	-0.15	-0.04	0.07
Rotifera spp.	-0.14	0.03	0.29**
Nauplii	-0.16	0.04	0.13
Copepodites	0.03	-0.08	-0.09
Cladocera	0.01	0.14	0.27**
Total zooplankton	-0.13	0.06	0.28**

Table 2-14. Correlations between herring larvae and potential zooplankton prey captured in potential tidal freshwater habitats of the Pamunkey River, Virginia, in the vicinity of Cumberland Thoroughfare (* $\alpha<0.05$, ** $\alpha<0.01$).

Potential Zooplankton Prey	River herring prolarvae	Gizzard shad prolarvae	Preflexion larvae	Postflexion larvae
Keratella spp.	-0.18	0.38**	0.28*	0.32*
Rotifera spp.	-0.16	0.35**	0.33*	0.27*
Nauplii	0.05	0.67**	0.35**	0.12
Copepodites	-0.05	0.24	0.04	-0.07
Cladocera	-0.13	0.24	0.08	0.05
Total zooplankton	-0.18	0.41**	0.34*	0.32*

PART III. Analysis of age and growth of herring larvae using the otolith increment method.

SUMMARY

1. Increment deposition in otoliths of river herring is suggested to occur daily. Apparent non-daily deposition can arise if increment spacing is less than the resolution afforded by light microscopy.

2. The mean diameter of the nucleus of river herring otoliths is 19μ (95% CI: 16μ to 22μ).

3. Growth of river herring larvae cultured in 1990 under non-suboptimal conditions was linear for about 12 to 14 days after hatching and appeared to accelerate after this age. Accelerated growth may have been associated with flexion of the caudal fin which increases the ability of larvae to pursue and capture prey.

4. When examining otoliths of field collected specimens, grinding and polishing of subsamples of postflexion larvae may be necessary to determine whether increments are "lost" near the edge of otoliths when viewed as whole mounts using light microscopy. It may also be necessary to examine subsamples of river herring preflexion larvae captured early in the spawning season (when conditions for growth are poorest) using electron microscopy to determine whether ages of these larvae are underestimated and whether growth rates are overestimated based on results from light microscopic examination.

PART III. Analysis of age and growth of herring larvae using the otolith increment method.

Objective A: Validate the rate of increment deposition in otoliths of known age alewife and blueback herring larvae.

Objective B: Determine whether growth rates differed between alewife and blueback herring larvae captured at different locations in the tidal freshwater reach of the Pamunkey River and between seasons.

INTRODUCTION

Differential growth and mortality of larval fish cohorts have been hypothesized as factors which significantly influence the variability of recruitment between year classes (Rothschild 1986). The identification of increments in otoliths of larval fishes (Pannella 1971) provides a method for the investigation of the dynamics of larval populations and of events that occur during the early life history. For example, using the otolith increment method to identify cohorts, Methot (1983) demonstrated that abundant cohorts of larvae may not provide the greatest contribution to the year class.

Under optimal conditions, otolith growth occurs through the daily, incremental deposition of the calcium carbonate - protein matrix (Brothers 1981; Campana and Neilson 1985). Some studies have suggested that non-daily formation can occur under extreme, sub-optimal conditions, but this may be a consequence of limited resolution of narrowly spaced rings rather than a disruption in otolith growth (Jones 1986; Jones and Brothers 1987).

Daily increment deposition has been documented to occur in otoliths of larvae and juveniles of a variety of clupeid species, including gizzard shad (Davis et al. 1985), Atlantic menhaden (Simoneaux and Warlen 1987), Atlantic herring (Geffen 1982; Messieh et al. 1987), and Pacific herring (McGurk 1984). Savoy and Crecco (1987) have documented daily increment deposition in otoliths of larval American shad. Essig and Cole (1986) suggest that increment deposition is daily in otoliths

of alewife larvae, and O'Rear (1983) has suggested that daily increment deposition occurs in otoliths of blueback herring larvae.

Validation of the relationship between age (in days) and otolith increment deposition is a critical component of age and growth studies of larval fishes (Beamish and McFarlane 1983; Geffen 1987). Of the methods available to validate otolith increment deposition rates, using larvae of known age is considered to be the best alternative (Geffen 1987). Prior to application of the otolith increment method to larvae captured in the field-study component of this project, it was necessary to develop expertise in preparing and analyzing larval otoliths and to test the hypothesis of daily increment deposition. This was accomplished using river herring larvae reared from eggs during the 1990 and 1991 spawning seasons (see Appendix B). The second objective of this study was not accomplished in the time allotted, but is a component of the dissertation research conducted by the primary author.

METHODS

Otoliths were dissected from a subsample of alewife and blueback herring larvae cultured from eggs during the 1990 and 1991 spawning seasons and measured prior to fixation (see Appendix B). Otoliths were dissected and mounted on glass slides following the general protocol outlined by Secor et al. (1991). To facilitate observation of otoliths during dissection, specimens were cleared in 1% potassium hydroxide (Brothers 1987). Larvae were transferred to water after clearing. To dissect otoliths, a larva was positioned on a clean microscope slide and was kept moist in water. Otoliths were dissected using microprobes constructed from insect pins set in dowel rod. Otoliths were then cleaned in either 3% hydrogen peroxide or 5% bleach to remove adhering tissue. Otoliths were air dried for at least 24 hours prior to being mounted on glass microscope slides using cyanoacrylate adhesive. Mounted otoliths were covered with histological-grade mounting medium

(Accu-mount, Baxter Scientific Co.) and a coverslip to enhance visibility of the increments. Nylon sewing thread was used to support the coverslip to prevent crushing the otoliths as the mounting media cured. Microscope slides used for mounting otoliths were etched on the reverse side to facilitate locating and identifying otoliths.

Otolith increments were enumerated using light microscopy under 40X magnification. Specimens were selected randomly for enumeration; the left otolith was used for enumeration if increments could be counted, otherwise the right otolith was used. All otoliths were evaluated, and the procedure was repeated. All otoliths were analyzed three times, after which the number of increments counted for each otolith were compared. Otoliths with increment counts that did not vary by more than two were accepted for analysis. Otoliths with increment counts that differed by more than two were randomized and enumerated again. The number of increments for each of these otoliths were compared and the count that deviated most was excluded. Otoliths were accepted for further analysis if the remaining increment counts did not differ by more than two; otoliths with increment counts that differed by more than two were excluded. For those otoliths accepted for analysis, the mean increment count (rounded to the nearest integer) was used. A small number of otoliths were evaluated under higher power using the Optimas pattern recognition system to measure increment widths, and the maximum and minimum diameters of otoliths and their nuclei.

Regression analysis (SPSS-X) was used to estimate the slopes of the relationships between otolith increment count and age for each group of larvae. The age of first increment deposition was estimated as the X-intercept of the regression line. The mean nucleus diameter was estimated from measurements on otoliths prior to deposition of the first increment dissected from late-stage prolarvae prior to complete yolk absorption.

RESULTS AND DISCUSSION

Otolith preparations.

Otoliths from a total of 89 cultured larvae were dissected for this study. Otoliths from 29 larvae were excluded for one or more reasons, including inconsistent increment counts between readings, inability to observed and enumerate increments due to adhering tissue or to being uncovered by an air bubble or air pocket, and misidentification of specimens. The greatest problem encountered in the enumeration of increments was incomplete removal of macular tissue from the otoliths. Otoliths were washed in 3% hydrogen peroxide or 5% sodium hypochlorite (bleach), but these solutions were not able to remove tissue in all cases. Secor et al. (1991) suggest using undiluted bleach to remove tissue.

Another significant problem was the formation of air bubbles or air pockets beneath the coverslip as the mounting medium cured. Air bubbles or pockets formed in many of the preparations; although, in most cases, these did not interfere with enumeration of otolith increments. Increments usually were not visible when otoliths were uncovered by an air bubble or pocket, but, in a few cases, increments were visible in larger otoliths not covered by mounting medium. Air pockets formed when both low- and high-viscosity mounting media were used. The underlying problem is suggested to have been the diameter of the nylon thread which appears to raise the coverslip enough to allow the mounting medium to contract while curing. Secor et al. (1991) suggest using strands of hair to support the cover slip.

Increment deposition.

Results of regression analyses for each group of cultured larvae were significant (Table 3-1). The results support the hypothesis of daily increment deposition for the 1990 river herring and 1991 blueback herring groups, with first increment formation occurring at one day

after hatching (Fig. 3a and 3b, respectively). Non-daily deposition of increments appears to have occurred in larvae of the 1991 alewife group (Fig. 3c) which is suggested to be a consequence of poor growth conditions experienced during the early postlarvae stage. The mean estimated daily growth increment for the cultured alewife larvae during the first several days following yolk sac absorption was 0.2 mm compared to the mean daily growth increment of 0.5 mm for blueback herring during the same developmental phase (see Appendix B). Greater growth of blueback herring during the early postlarvae stage promoted wider spacing between increments which improved the accuracy of the increment counts compared to the increment counts from alewife otoliths. Suboptimal conditions experience by alewife larvae during early life are suggested to have caused the deposition of narrow-spaced increments that were below the resolution of light microscopy (Jones and Brothers 1987). Growth of older blueback herring larvae was also reduced causing increment widths to become narrower. However, it is suggested that spacing between increments was less affected since body reserves would be mobilized to maintain growth as the amount of ingested food decreased.

The 1990 river herring group appears to have been reared under conditions favorable to growth throughout development (see Appendix B). Of the three groups of cultured larvae, the results from the 1990 river herring group are suggested to most accurately reflect the deposition of increments in otoliths under non-suboptimal conditions. Underestimation of increments in larger otoliths from older postlarvae may be a significant source of potential error; therefore, it may be necessary to grind and polish otoliths of postflexion larvae to obtain reliable estimates of age from increment counts. It may also be necessary to validate increment counts in subsamples of river herring preflexion larvae captured early in the spawning season (when conditions for growth are poorest) using electron microscopy to determine whether ages are

underestimated and whether growth rates are overestimated based on results using light microscopy.

Nucleus diameter.

Otoliths from six late-stage prolarvae from the 1990 river herring group were measured to estimate the nucleus diameter prior to deposition of the first increment. Diameters of observed minor and major axes of the otoliths were measured and averaged. The average diameter of the six otoliths was 19u (95% CI: 16u to 22u), and provides a guide to facilitate identification of the nucleus and the location of the first increment.

Retrospective growth curves.

Figures 3-2a to d illustrate individual retrospective growth curves for several larvae from the 1990 river herring group. The results demonstrate consistency in the growth of these larvae and show that growth was approximately linear during early life following yolk sac absorption. At about 13 to 14 days after hatching, however, growth appears to have increased, possibly in association with flexion which would increase the ability of larvae to pursue and capture prey. Underestimation of increments near the edge of larger otolith could result in the appearance of accelerated growth. Retrospective growth curves also provide a method to identify possible errors in the identification of increments by showing zones of apparent growth acceleration or deceleration not consistent with adjacent increments (Fig. 3-2b to 3-2d).

LITERATURE CITED

Beamish, R. J. and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society 112: 735-743.

Brothers, E. B. 1981. What can otolith microstructure tell us about daily and subdaily events in the early life history of fish? Rapports et Proces-Verbaux des Reunions, Consel International pur l'Exploration de la Mer 178: 393-394.

Brothers, E. B. 1987. Methodological approaches to the examination of otoliths in aging studies, pages 319-330. *In*: R. C. Summerfelt and G. E. Hall (editors), Age and growth of fish. Iowa State Univ. Press, Ames, Iowa. 544 p.

Campana, S. E. and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42: 1014-1032.

Davis, R. D., T. W. Storck and S. J. Miller. 1985. Daily growth increments in the otoliths of young-of-the-year gizzard shad. Transactions of the American Fisheries Society 114: 304-306.

Essig, R. J. and C. F. Cole. 1986. Methods of estimating larval fish mortality from daily increments in otoliths. Transactions of the American Fisheries Society 115: 34-40.

Geffen, A. J. 1987. Methods of validating daily increment deposition in otoliths of larval fish, pages 223-240. *In*: R. C. Summerfelt and G. E. Hall (editors), Age and growth of fish. Iowa State Univ. Press, Ames, Iowa. 544 p.

Jones, C. 1986. Determining age of larval fish with otolith increment technique. Fishery Bulletin, U.S. 84: 91-103.

Jones, C. and E. B. Brothers. 1987. Validation of the otolith increment aging technique for striped bass, *Morone saxatilis*, larvae reared under suboptimal feeding conditions. Fishery Bulletin, U.S. 85: 171-178.

McGurk, M. D. 1984. Ring deposition in otoliths of larval Pacific herring, *Clupea harengus pallasi*. Fishery Bulletin, U.S. 84: 13-26.

Messieh, S. N., D. S. Moore and P. Rubec. 1987. Estimation of age and growth of larval Atlantic herring as inferred from examination of daily growth increments of otoliths, pages 433-442. *In*: R. C. Summerfelt and G. E. Hall (editors), Age and growth of fish. Iowa State Univ. Press, Ames, Iowa. 544 p.

Methot, R. D., Jr. 1983. Seasonal variation in survival of larval northern anchovy, *Engraulis mordax*, estimated from the age distribution of juveniles. Fishery Bulletin, U.S. 81: 741-750.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173: 1124-1127.

O'Rear, C. W. 1983. A study of river herring spawning and water quality in Chowan River, N.C. Completion Report, AFC-17, East Carolina University, Greenville, N.C. 31 p.

Rothschild, B. J. 1986. Dynamics of Marine Fish Populations. Harvard University Press, Cambridge. 277 p.

Savoy, T. F. and V. A. Crecco. 1987. Daily increments on the otoliths of larval American shad and their potential use in population dynamics studies, pages 413-432. *In*: R. C. Summerfelt and G. E. Hall (editors), Age and growth of fish. Iowa State Univ. Press, Ames, Iowa. 544 p.

Secor, D. H., J. M. Dean, and E. H. Laban. 1991. Manual for otolith removal and preparation for microstructural examination. Publication 1991-01, Belle W. Baruch Intitute for Marine Biology and Coastal Research. 85 p.

Simoneaux, L. F. and S. M. Warlen. 1987. Occurrence of daily growth increments in otoliths of juvenile Atlantic menhaden, pages 443-452. *In*: R. C. Summerfelt and G. E. Hall (editors), Age and growth of fish. Iowa State Univ. Press, Ames, Iowa. 544 p.

Table 3-1. Summary of regression analyses of increment deposition with age in otoliths of river herring larvae cultured during the 1990 and 1991 spawning seasons.

N	Slope	r^2	Standard Error	X-intercept	ANOVA *F*	ANOVA P
			1990 River herring			
26	1.02	0.92	1.58	0.6	288.1	<0.001
			1991 Blueback herring			
18	0.84	0.97	1.63	1.2	523.6	<0.001
			1991 Alewife			
16	0.70	0.92	2.05	5.5	153.7	<0.001

Figure 3-1a. Linear regression relationship between otolith increment number and age of river herring larvae (species unidentified) cultured during the 1990 spawning season.

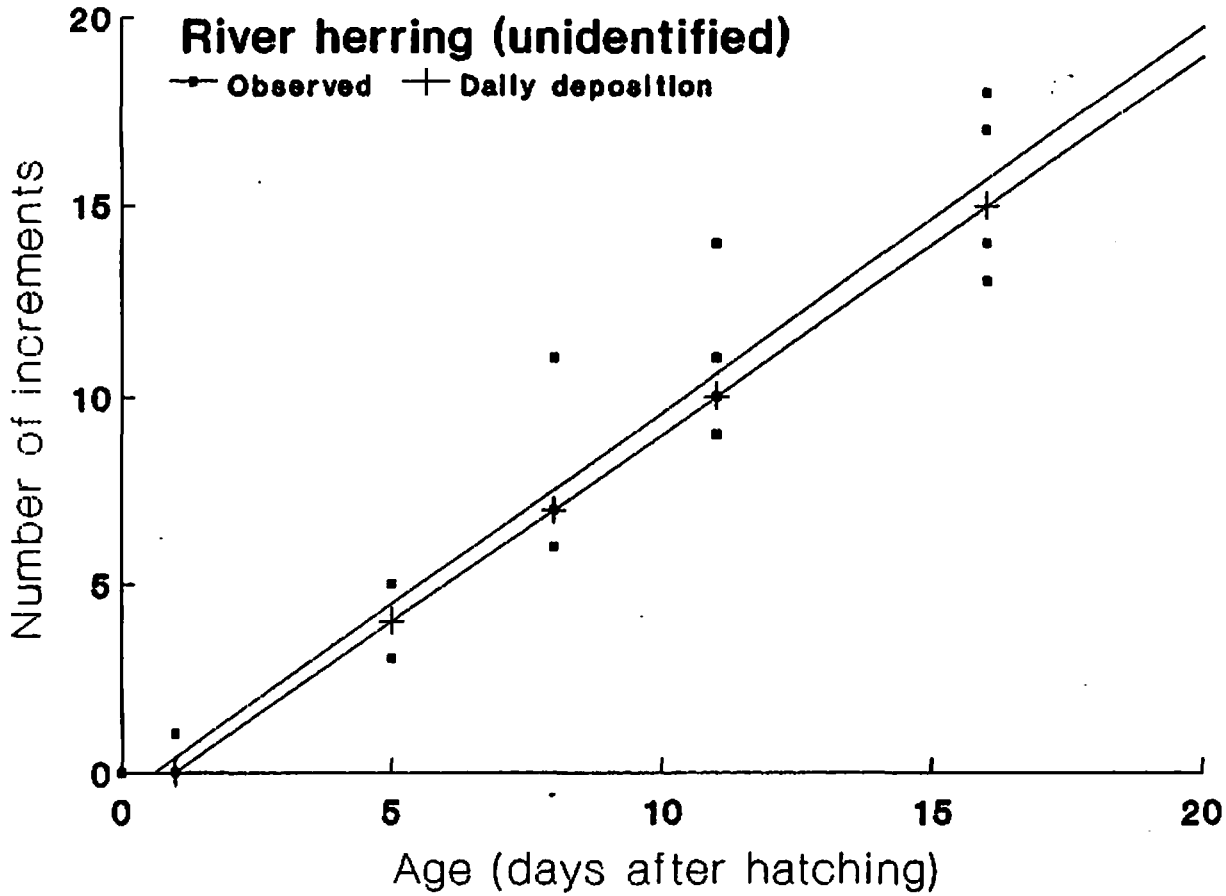

River herring (unidentified)
Observed
Daily deposition
Number of increments
Age (days after hatching)
0
5
10
15
20

Figure 3-1b. Linear regression relationship between otolith increment number and age of blueback herring larvae cultured during the 1991 spawning season.

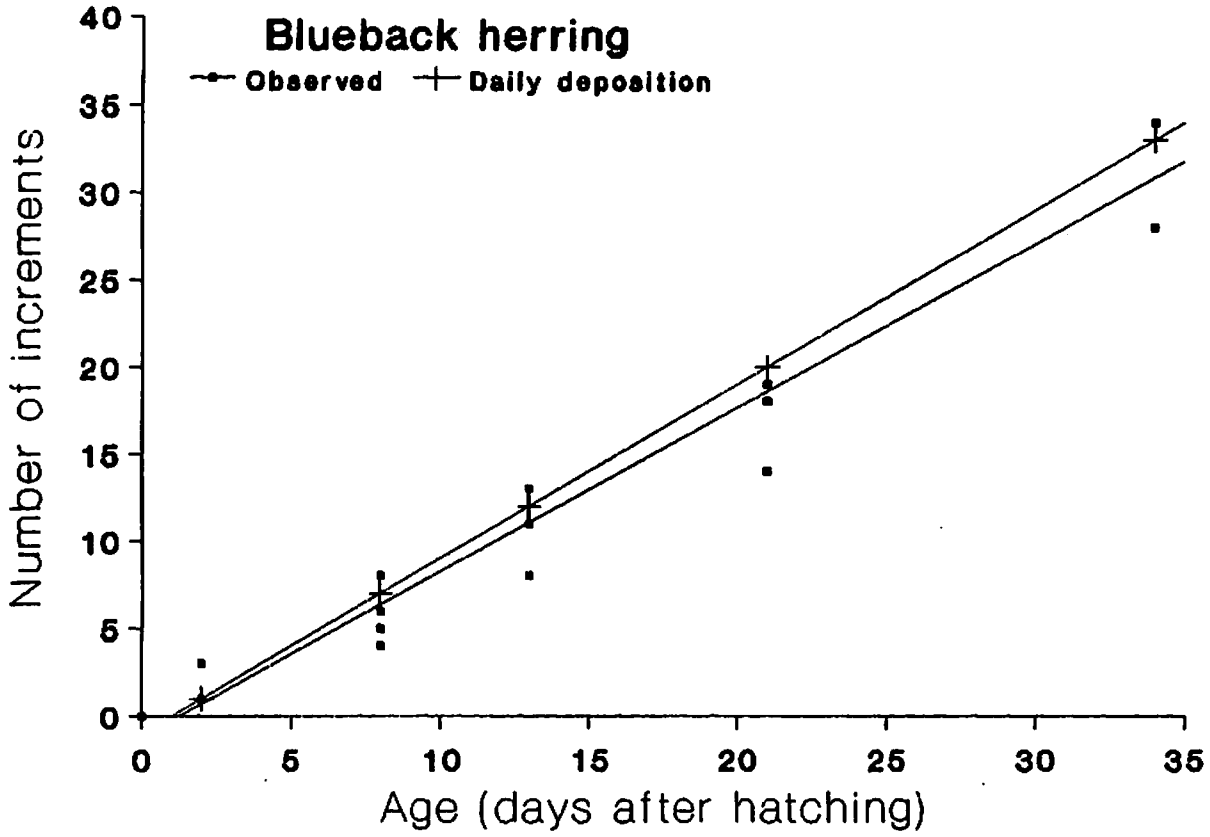

Blueback herring
Observed
Daily deposition
Number of increments
Age (days after hatching)
0
5
10
15
20
25
30
35
40

Figure 3-1c. Linear regression relationship between otolith increment number and age of alewife larvae cultured during the 1991 spawning season.

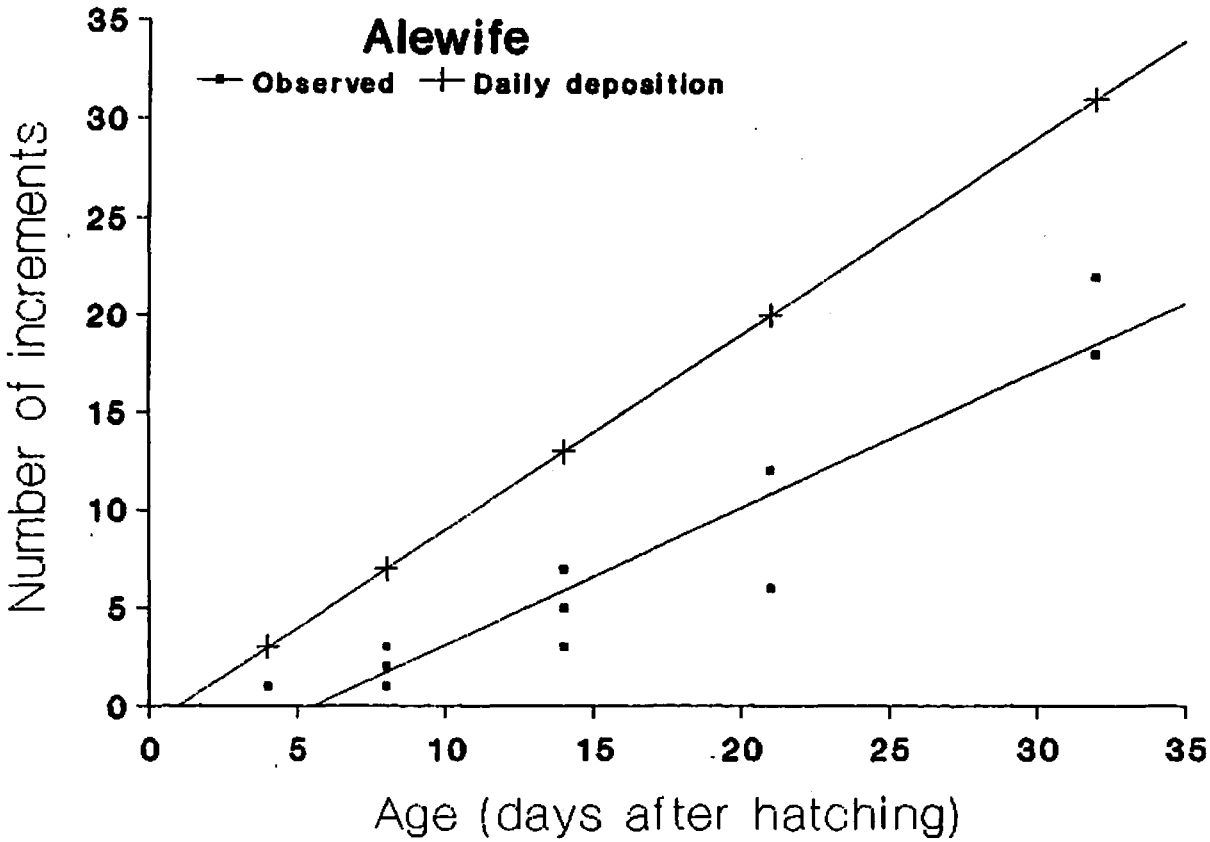

Alewife
Observed
Daily deposition
Number of increments
Age (days after hatching)
0
5
10
15
20
25
30
35

Figure 3-2a. Individual retrospective growth curve for Sample 110590/01. Otolith length is the radius from the center of the primordia to the edge of the otolith along the maximal growth radius. Increment 0 represents the edge of the nucleus.

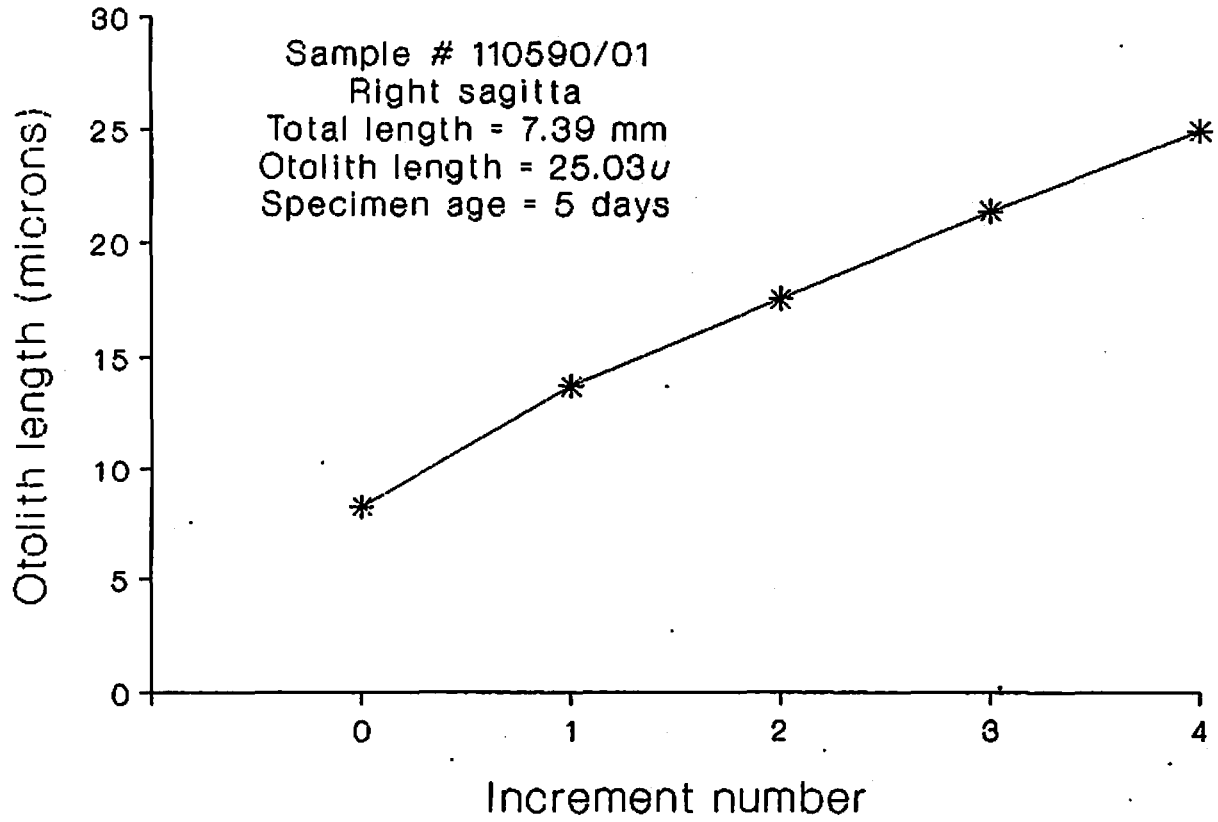

Sample # 110590/01
Right sagitta
Total length = 7.39 mm
Otolith length = 25.03u
Specimen age = 5 days
Otolith length (microns)
30
25
20
15
10
5
0
0
1
2
3
4
Increment number

Figure 3-2b. Individual retrospective growth curve for Sample 220590/03. Otolith length is the radius from the center of the primordia to the edge of the otolith along the maximal growth radius. Increment 0 represents the edge of the nucleus. The arrow identifies a possible subdaily increment misclassified as a daily increment.

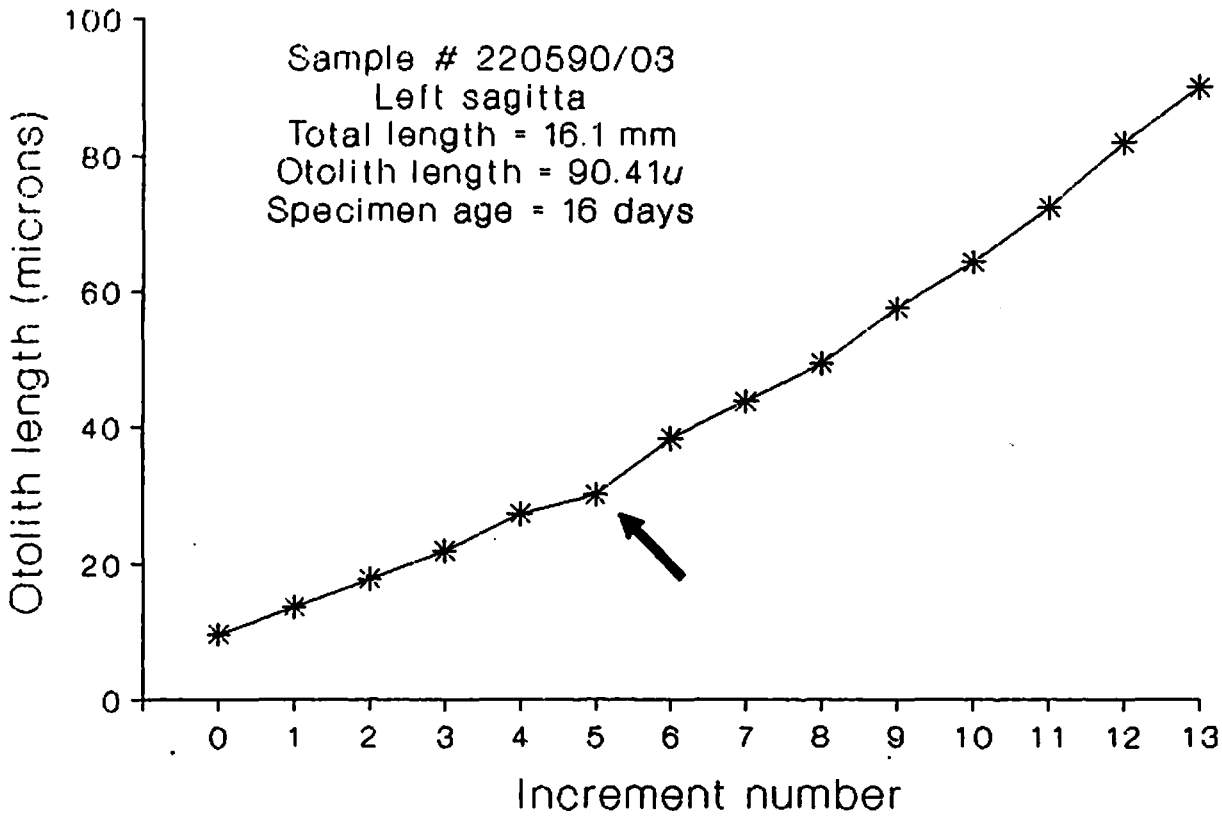

Sample # 220590/03
Left sagitta
Total length = 16.1 mm
Otolith length = 90.41u
Specimen age = 16 days
Otolith length (microns)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Increment number

Figure 3-2c. Individual retrospective growth curve for Sample 220590/04. Otolith length is the radius from the center of the primordia to the edge of the otolith along the maximal growth radius. Increment 0 represents the edge of the nucleus. The arrows identify possible subdaily increments misclassified as daily increments.

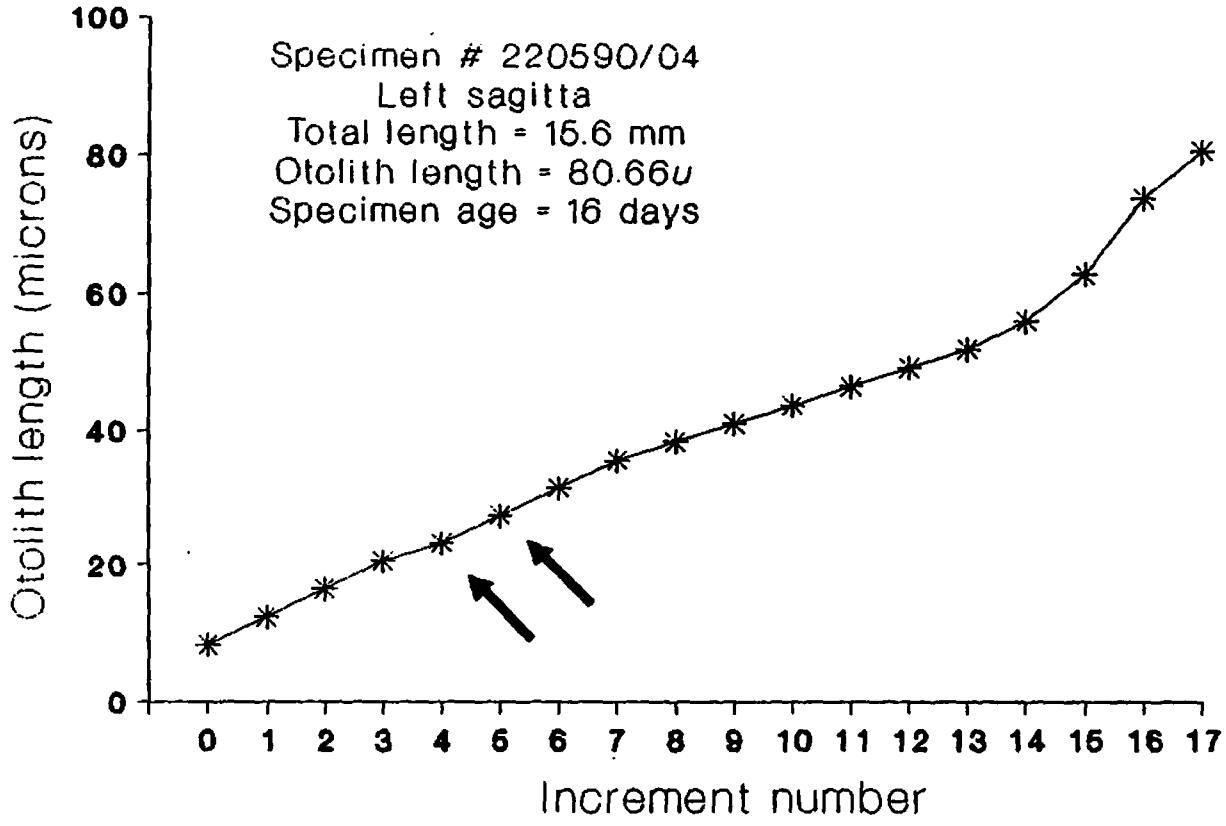
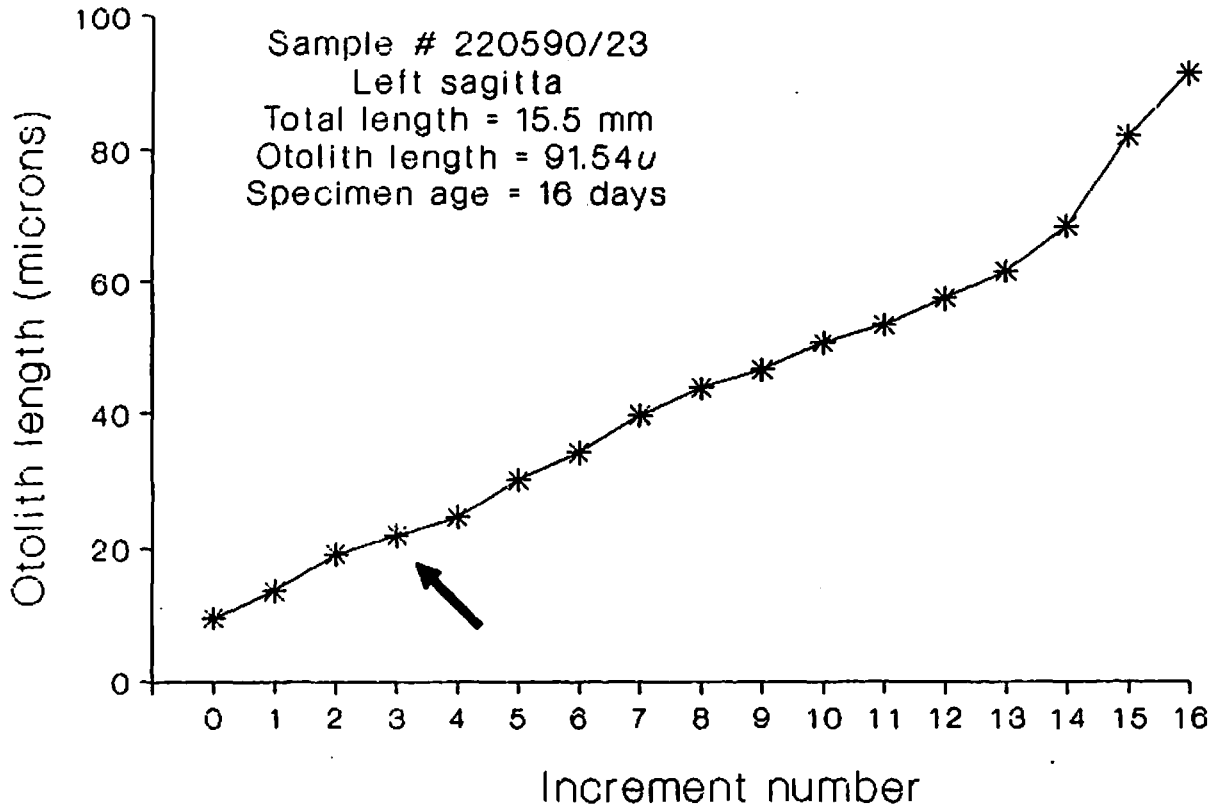

Specimen # 220590/04
Left sagitta
Total length = 15.6 mm
Otolith length = 80.66u
Specimen age = 16 days
Otolith length (microns)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Increment number

Figure 3-2d. Individual retrospective growth curve for Sample 220590/23. Otolith length is the radius from the center of the primordia to the edge of the otolith along the maximal growth radius. Increment 0 represents the edge of the nucleus. The arrow identifies a possible subdaily increment misclassified as a daily increment.


Sample # 220590/23
Left sagitta
Total length = 15.5 mm
Otolith length = 91.54u
Specimen age = 16 days
Otolith length (microns)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Increment number

APPENDIX A.

A Method for Rearing Alewife (*Alosa pseudoharengus*) and Blueback Herring (*A. aestivalis*) Postlarvae Using a Continuous-flow System

A Method for Rearing Alewife (*Alosa pseudoharengus*) and Blueback Herring (*A. aestivalis*) Postlarvae Using a Continuous-flow System

by

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1. This study was conducted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Alewife and blueback herring postlarvae are difficult to rear; despite many attempts, few researchers have been able to induce larvae to begin active feeding in aquaria. An alternative method for rearing alewife and blueback herring postlarvae using a continuous-flow system is described. Embryos and prolarvae were reared in an aquaria system using recirculated water and were transferred to rearing tanks of the continuous-flow system prior to complete yolk absorption. Fungal infestation was a significant impediment to egg hatching in the enclosed aquaria system. Fungal infestation occurred in water from most of the sources used to incubate eggs; however, fungal growth was inhibited in water from Herring Creek, Virginia, and hatching of both alewife and blueback herring eggs was consistently high. Postlarvae were reared in the continuous-flow system using water drawn from the Pamunkey River, Virginia. In 1990, a total of 51 postlarvae were recovered, and a single larva was recovered 24 days after hatching. In 1991, 280 alewife larvae and 521 blueback herring larvae were recovered. Alewife larvae were reared for 32 days after hatching and blueback herring postlarvae were reared for 37 days after hatching. Comparisons are made between this system and a continuous-flow system in which larvae failed to survive so that possible factors of potential importance in the design of a laboratory system for the culture of these species might be identified.

INTRODUCTION

Many attempts to rear postlarvae of alewife (*Alosa pseudoharengus*) and blueback herring (*A. aestivalis*), collectively referred to as river herring, have been unsuccessful (Mansueti 1956; Norden 1967, 1968; Cianci 1969; Davis et al. 1972; Loesch and Kriete 1976; Lam and Roff 1977; Schubel et al. 1977; Kellogg 1982; Klauda and Palmer 1987). The inability of river herring larvae to bridge the transition from endogenous to exogenous nourishment appears to be the principal factor that inhibits successful culture. The availability of appropriate types of prey of suitable size and at a suitable density at the time of first-feeding is one of many potential factors that may inhibit successful culture of fish larvae (Blaxter 1968; Houde 1973; Kinne 1977), but it is critical since larvae must feed to grow.

Wild zooplankton often is used to provide food for larval fishes. Heinrich (1981) successfully reared alewife larvae to the juvenile stage in a closed system utilizing wild zooplankton and supplemented with brine shrimp (*Artemia salina*) nauplii beginning 15 days after hatching. Wild zooplankton potentially offers a wide variety of prey types from which larvae may select, but drawbacks include unknown, variable, and uncontrolled composition and quality, the introduction of pathogens, parasites or predators, and fluctuating availability (Kinne 1977). Cultured prey provides a controlled, reliable food source and avoids disadvantages associated with using wild zooplankton (Kinne 1977). However, culturing prey or collecting zooplankton in sufficient quantity to support large numbers of river herring larvae for experimental studies can be difficult and labor-intensive, and controlled intensive culture of many types of potential prey often requires suitable facilities. *Artemia* nauplii are extensively used as prey for fish larvae and are relatively easy to culture, but they are too large for larvae of many species of fishes, especially those of marine origin, to utilize at first-feeding (Houde 1973).

Substantial investment of time, space, and labor to culture potential prey or to collect zooplankton may not be feasible or practical. An alternative approach to rearing alewife and blueback herring larvae is necessary. A continuous-flow system was developed to rear the larvae because intensive collection of zooplankton was not possible. The system was designed to enable constant delivery of potential prey to rearing tanks of sufficient volume to promote larval development and minimize adverse effects associated with rearing container size (Kinne 1977; Theilacker 1980; Barahona-Fernandes and Conan 1981).

METHODS

Running-ripe alewife and blueback herring were collected on spawning grounds in tidal freshwater areas of eastern Virginia. Alewife were collected from Massaponax Creek, a tributary of the Rappahannock River near Fredericksburg, Virginia. Blueback herring were obtained in tidal freshwater of the Pamunkey River main-stem in 1989 and from Herring Creek, a tributary of the James River near Williamsburg, Virginia, in 1991. River herring eggs from a natural spawn were collected from Herring Creek in 1990. The taxonomic identity of the adults was verified using peritoneal pigmentation; alewife with unpigmented peritoneum and blueback herring with black peritoneum were used to obtain eggs and milt (Loesch 1987). River herring with mottled or incomplete peritoneal pigmentation were not used because of questionable taxonomic identity. Eggs were fertilized following the dry-method in a shallow glass dish (Piper et al. 1982), were poured into 333μ mesh nylon screen sieves and rinsed to remove excess milt, and were transported in a shallow layer of water in coolers to the rearing facility. The aquaria system for rearing embryos and prolarvae and the continuous-flow system were located approximately 17 nautical miles upriver from West Point, Virginia, near the upper limit of the Pamunkey

River estuary (Van Engel and Joseph 1968) within the historical nursery area of juvenile *Alosa*.

Water for rearing eggs and prolarvae was obtained from several sources: the Pamunkey River, Herring Creek, and Massaponax Creek. Pamunkey River water was filtered through a 0.5μ polyethylene filter in 1989 and through a 5.0μ polyethylene filter in 1990. Water from Herring Creek and Massaponax Creek was filtered through a 35μ sieve. All water used for incubating eggs was treated with aquarium-grade fungus inhibitor which was ineffective.

Eggs were reared in closed, recirculating systems. In 1989, embryos and prolarvae were reared in a 37.8 L rectangular aquarium with water recirculated using an aquarium pump with non-adjustable flow. The output from this pump was split into two lines, one leading to a filter containing glass wool, charcoal, and ammonia adsorbent, and the other was directed onto the eggs to facilitate oxygenation. A hatching system was developed subsequent to the 1989 season that incorporated two 37.8 L aquaria for rearing eggs and larvae and a third 37.8 L aquarium used as a reservoir. Circulation was provided using a power filter (Fluval Model 303®). Water was circulated through a main-line to maintain high flow velocity and prevented formation of air bubbles in the siphon leading to the power filter. Slow circulation promoted air bubble formation that could interrupt water flow. Water diverted from the main-line to aquaria containing eggs and prolarvae was regulated using a tubing clamp to provide a steady flow. Aquarium circulation was enhanced using a pump with adjustable flow. Overflow from each aquarium drained into the reservoir through an outlet located near the top in one end-wall. The area used for rearing embryos and larvae in each aquarium was separated from the overflow outlet using 333μ nylon screen.

Figure 1 illustrates the water intake and delivery components of the continuous-flow system, and Fig. 2 illustrates the rearing tanks and overflow assembly. A 1000-GPH sump pump suspended within a capped 208 L

plastic barrel perforated with 6.25 mm diameter holes was used to pump water from the Pamunkey River. The barrel was suspended from a floating platform to provide constant head pressure. Vinyl-coated chicken wire surrounded the platform perimeter to a depth of about 1 m. Water was delivered through standard 15.6 mm diameter hose into the bottom of a sealed 208 L plastic barrel half-filled with pea-gravel which served as a coarse filter and settling tank. Flow from an outlet near the top of the barrel split into two lines which drained into 300μ mesh nylon filter socks suspended in capped 208 L plastic barrels. The filtrate from each barrel drained into larval rearing tanks.

Two fiber-glass tanks were used to rear larvae. Each had a perimeter depth of 80 cm, a central depth of 100 cm, and a diameter of 120 cm. Water depth at the center varied from 91 cm to 96 cm depending on the clogging of wire screen covering outlet holes at the base of a standpipe sleeve. Each tank was estimated to contain approximately 1050 L (approximately 1 m^3). A bluegreen vinyl tank liner was used in each tank to provide a contrasting background to facilitate foraging by larvae (Kinne 1977).

The overflow assembly for each tank (Fig. 2) consisted of a 50 mm diameter polyvinyl chloride (PVC) pipe, 91 cm in height, inserted through a 100-to-50 mm PVC reducing adapter, with a 100 mm diameter PVC pipe, 96 cm in height, as an outer sleeve. The inner overflow standpipe fit into the drain adapter of the tank. The outer sleeve had 50 mm diameter holes near the base covered with 300μ wire mesh screen which forced water to drain from the tank bottom facilitating circulation and oxygenation. The reducing adapter prevented larvae from being drawn under the sleeve and flushed from the system.

RESULTS AND DISCUSSION

In 1990, a total of 51 larvae were recovered from the rearing tanks, a single larva was sampled at 24 days after hatching. In 1991,

280 alewife larvae and 521 blueback herring larvae were recovered. Alewife larvae were reared for 32 days after hatching and blueback herring larvae were reared for 37 days after hatching.

Fungal infestation was a significant constraint to successful egg hatching. Extensive infestation occurred when eggs were incubated in water from Massaponax Creek +wd in Pamunkey River water filtered to 5.0μ. Fungal growth did not occur on eggs incubated in Pamunkey River water filtered to 0.5μ or in Herring Creek water. The hatching success of eggs incubated in Herring Creek water was consistently high (visually estimated to be over 90%). Characteristics of Herring Creek water may derive from the decomposition and leaching of accumulated plant litter in Harrison Lake which is drained by Herring Creek. Tannin in Herring Creek water may have been important in limiting fungal growth.

Fungal infestation of eggs has been a significant problem in the rearing of alewife and blueback herring embryos. Davis et al. (1972) reported that they were able to control fungal infestation by using either Wardley's fungus remedy at the recommended dosage or malachite green (0.05-0.1 ppm), and they reported that the hatching rate of alewife and blueback herring prolarvae varied from 0 to over 50% which was attributed to variability in the ripeness of eggs at the time of fertilization. However, adverse influence from chemical treatment to control fungus cannot be discounted (Meyer and Jorgenson 1983). Schubel et al. (1977) also reported significant fungal infestation during their attempts to rear alewife and blueback herring larvae. Krise et al. (1986) suggest that bacterial, fungal, or viral pathogens are carried in or on the adhesive layer and that the incidence of pathogenic infestation may be reduced by its removal.

Culturing zooplankton prey or collecting wild zooplankton to feed larval fishes require significant investment in labor, time, and space which may not always be possible. Such circumstances led to the development of the continuous-flow system described herein. Wild

zooplankton entrained in water from the Pamunkey River established and supplemented prey populations in the large-volume rearing containers. Schubel et al. (1977) reported using a continuous-flow system, described by Schiemer and Schubel (1974), in their attempt to rear alewife and blueback herring larvae. Although that system was located in an area rich in zooplankton (the upper Potomac River estuary), larvae failed to survive (Schubel et al. 1977).

Comparison between our and the Schiemer-Schubel system may provide information on system design criteria valuable for future investigations. Differences between the two systems pertain to characteristics of the water intake, the location of the intake in the water column, the method of pumping entrained water, and rearing container size. Water temperature was controlled in the Schiemer-Schubel system but was not controlled in our system.

The water intake of the Schiemer-Schubel system consisted of 1.6 mm mesh screen with an area of about 0.1 m^2 maintained at a fixed depth of 0.6 m above a sandy bottom, water was entrained by suction, and water temperature was regulated (Schiemer and Schubel 1974). Larvae were maintained in relatively small holding containers that floated in larger aquaria (Schubel et al. 1977). The water intake unit for our system consisted of a 208 L plastic barrel perforated with 6.4 mm diameter holes suspended from a floating platform to maintain constant intake pressure. The barrel was maintained at about 0.3 m below the surface and the pump was about the 0.3 m below the barrel lid.

Relative density and composition of zooplankton entrained by the Schiemer-Schubel system were not reported (Schubel et al. 1977), and the potential prey available for larvae is not known. Based on the design of their system, few zooplankton may have been entrained or zooplankton may have been killed as they passed through the intake screen and were of no value to the larvae as food. Relatively small mesh openings could have increased the acceleration of water close to the screen, and the

small surface area of the intake might have enabled zooplankters to escape and leave the immediate vicinity. Since the system relied on suction to entrain water, the intake velocity may have been relatively low which also could increase the chance for escape. The sump pump used for the intake of our system, in comparison, created relatively high pressure and screens which might kill zooplankton were not used. With the pump enclosed in a barrel of relatively large volume perforated with relatively large holes (four times larger than the mesh used in the Schiemer-Schubel system), the likelihood that potential prey would enter the vicinity of the pump intake was greatly enhanced. With inflow through the barrel surface, the likelihood that zooplankton would escape the pump and leave the area might also have been reduced.

Filter socks (300μ mesh) were used in our system to exclude larger zooplankton, including fish larvae, from the rearing tanks. Leslie and Timmins (1989) reported that a mesh size of 250μ retained 100 percent of the experimental catch in towed nets, but 6 percent of the catch was theoretically capable of being extruded. The possible introduction of herring larvae from the river into the rearing tanks cannot be excluded. Herring eggs with embryos were found adhered to the outer surface of the intake unit of the culture system on 7 June 1991 when the system was disassembled, suggesting that egg and larval uptake into the system was probable.

The distribution of zooplankton was not known in either of the locations where these systems were used, but the relative location of the intake in the water column might have been important. Diel variation in the distribution of zooplankton would influence the exposure of zooplankton to a system's intake, and both natural and artificial currents would interact with the intake pressure to affect the likelihood of zooplankton becoming entrained. The intake of the Schiemer-Schubel system was located over a sandy bottom (Schiemer and Schubel 1974) which suggests that it was located in an area

characterized by relatively rapid current that might have swept zooplankton past the intake before they could be entrained. The intake for our system was located near the surface and was subject to strong tidal currents; however, using a perforated barrel to collect and limit dispersal of zooplankton may have reduced the influence of these currents on zooplankton entrainment.

Large-volume larval rearing containers used in the present system appear to have facilitated the establishment of zooplankton prey populations and to have promoted growth of larvae similar to growth that could occur among natural populations. Growth rates of river herring larvae reared in these containers were similar to growth rates of bay anchovy *Anchoa mitchilli* larvae, 0.39 to 0.63 mm d^{-1}, reared in mesocosms in Chesapeake Bay (Cowan and Houde 1990). Large volume rearing containers may not be feasible for controlled experimentation, but they enable the culture of relatively large numbers of alewife and blueback herring larvae without intensive daily maintenance. It may then be possible to transfer older larvae at a size capable of ingesting *Artemia* nauplii to smaller aquaria for experimental study.

ACKNOWLEDGEMENTS

Special acknowledgement is made of the assistance provided by Bob and Rose Winsor who permitted the culture system to be set up on their property, and provided a storage room of their house for use as a field-laboratory. The authors also wish to thank Morris Roberts, George Pongonis, and Dexter Haven for their suggestions and critiques of preliminary system designs, and Morris Roberts for use of the large volume containers. Dr. Mike Castagna and Mr. Bruce Hill critically reviewed a preliminary draft of this manuscript.

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LITERATURE CITED

Barahona-Fernandes, M.H. and G. Conan. 1981. Daily food intake of reared larvae of the European seabass (*Dicentrarchus labrax* L.) statistical analysis and modelling. Rapports et Proces-Verbaux des Reunions, Consel International pur l'Exploration de la Mer 178:41-44.

Blaxter, J. H. S. 1968. Rearing herring larvae to metamorphosis and beyond. Journal of the marine biological Association, United Kingdom 48: 17-28.

Cianci, J. M. 1969. Larval development of the alewife, *Alosa pseudoharengus* Wilson, and the glut herring, *Alosa aestivalis* Mitchill. M.S. thesis, University of Connecticut, Storrs, Connecticut. 62 p.

Cowan, J. H., Jr. and E. D. Houde. 1990. Growth and survival of bay anchovy *Anchoa mitchilli* larvae in mesocosm enclosures. Marine Ecology - Progress Series 68: 47-57.

Davis, J., J. V. Merriner, W. J. Hoagman, R. A. St. Pierre and W. L. Wilson. 1972. Biology and utilization of anadromous alosids; Job 6: Development of culture technique. Annual Progress Report, AFC 7-2, United States Bureau for Commercial Fisheries.

Heinrich, J. W. 1981. Culture, feeding, and growth of alewifes hatched in the laboratory. Progressive Fish-Culturist 43: 3-7.

Houde, E. D. 1973. Some recent advances and unsolved problems in the culture of marine fish larvae. Proceedings of the World Mariculturist Society 3: 83-112.

Kellogg, R. L. 1982. Temperature requirements for the survival and early development of the anadromous alewife. Progressive Fish-Culturist 44: 63-73.

Kinne, O. 1977. Cultivation of animals: Research cultivation-Pisces, pages 968-1035. *In*: O. Kinne (editor), Marine Ecology: A comprehensive, integrated treatise on life in oceans and coastal waters, Vol. III, pt. 2. John Wiley and Sons, Chichester.

Klauda, R. J. and R. E. Palmer. 1987. Responses of blueback herring eggs and larvae to pulses of acid and aluminum. Transactions of the American Fisheries Society 116: 561-569.

Krise, W. F., L. Bulkowski-Cummings, A. D. Shellman, K. A. Kraus, and R. W. Gould. 1986. Increased walleye egg hatch and larval survival after protease treatment of eggs. Progressive Fish-Culturist 48: 95-100.

Lam, C. N. H. and J. C. Roff. 1977. A method for separating alewife *Alosa pseudoharengus* from gizzard shad *Dorosoma cepedianum* larvae. Journal of Great Lakes Research 3: 313-316.

Leslie, J. K. and C. A. Timmins. 1989. Double nets for mesh aperture selection and sampling in ichthyoplankton studies. Fisheries Research 7: 225-232.

Loesch, J. G. 1987. Overview of life history aspects of anadromous alewife and blueback herring in freshwater habitats. American Fisheries Society Symposium 1: 89-103.

Loesch, J. G. and W. H. Kriete, Jr. 1976. Biology and management of river herring and shad in Virginia. Completion Report, 1974-1976. National Marine Fisheries Service, Project Number AFC 8-1 to 8-3.

Mansueti, R. J. 1956. Alewife herring eggs and larvae reared successfully in lab. Maryland Tidewater News 13: 2-3.

Meyer, F. P. and T. A. Jorgenson. 1983. Teratological and other effects of malachite green on development of rainbow trout and rabbits. Transactions of the American Fisheries Society 112: 818-824.

Norden, C. R. 1967. Development and identification of the larval alewife, *Alosa pseudoharengus* (Wilson), in Lake Michigan. Journal of Great Lakes Research 10: 70-78.

Norden, C. R. 1968. Morphology and food habits of the larval alewife, *Alosa pseudoharengus* (Wilson), in Lake Michigan. Journal of Great Lakes Research 11: 103-110.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler and J. R. Leonard. 1982. Fish hatchery management. United States Fish and Wildlife Service, Washington, D.C.

Schiemer, E. W. and J. R. Schubel. 1974. Constant temperature water supply system. Special Report 39, Reference 74-9, Chesapeake Bay Institute, The Johns Hopkins University, Baltimore, Maryland.

Schubel, J. R., T. S. Y. Koo, and C. F. Smith. 1977. Thermal effects of power plant entrainment on survival of fish eggs and larvae: A laboratory assessment. Chesapeake Science 18: 290-298.

Theilacker, G. H. 1980. Rearing container size affects morphology and nutritional condition of larval jack mackerel, *Trachurus symmetricus*. Fishery Bulletin, U.S. 78: 789-791.

Van Engel, W. A., and E. B. Joseph. 1968. Characterization of coastal and estuarine fish nursery grounds as natural communities. Final Report, United States Bureau for Commercial Fisheries.

Figure 1. Water intake and delivery system for the continuous-flow culture system.

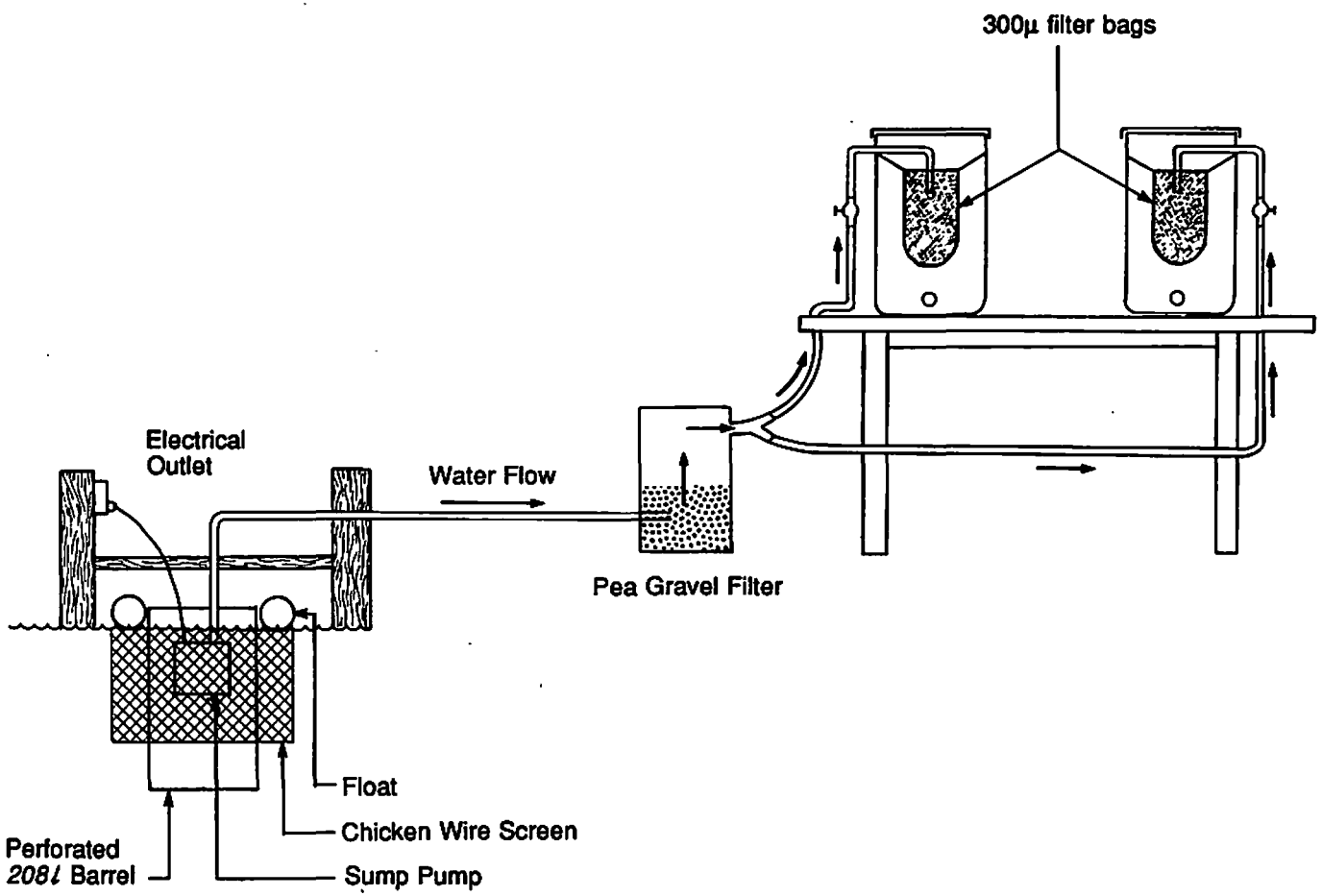
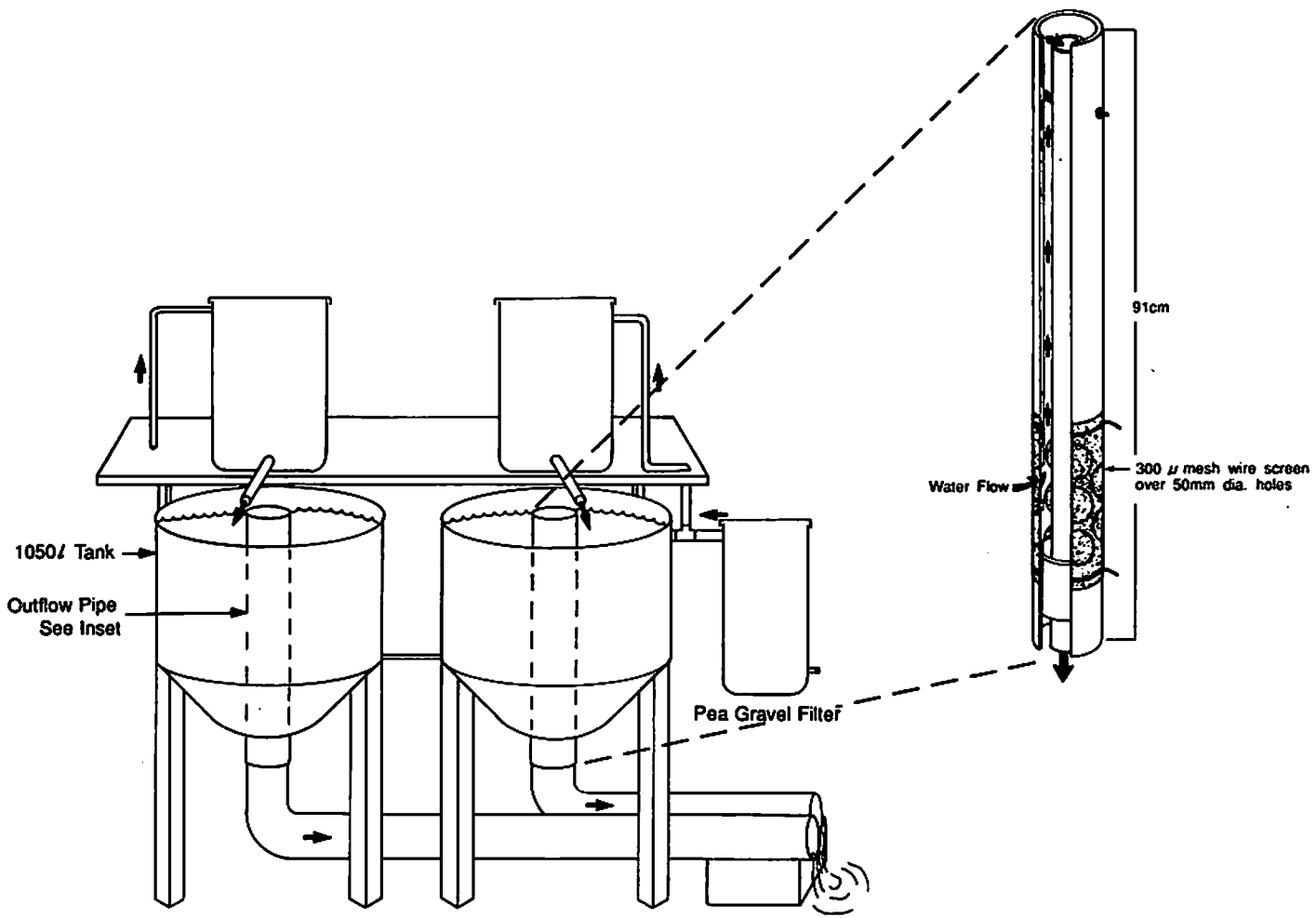

300µ filter bags
Electrical
Outlet
Water Flow
Pea Gravel Filter
Float
Chicken Wire Screen
Perforated
208ℓ Barrel
Sump Pump

Figure 2. Rearing tanks and overflow assembly for the continuous-flow culture system.


1050ℓ Tank
Outflow Pipe
See Inset
Pea Gravel Filter
91cm
Water Flow
300 μ mesh wire screen
over 50mm dia. holes

APPENDIX B.

The Culture of Alewife (*Alosa pseudoharengus*) and Blueback Herring (*A. aestivalis*) Postlarvae In a Continuous-flow System

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ABSTRACT

Alewife and blueback herring postlarvae are difficult to rear and maintain in aquaria. Presentation of zooplankton prey that stimulates active feeding appears to be the primary limitation to successful rearing of these species in the laboratory. Collecting and processing large quantities of wild zooplankton is labor-intensive, and culturing prey can be expensive, often requiring suitable facilities. An alternative approach to rearing these species beyond the transition to exogenous nourishment would be valuable for experimental studies.

This report documents the rearing of alewife and blueback herring postlarvae in a continuous-flow system. Growth of alewife and blueback herring larvae in 1990 and in 1991 differed significantly. This was most likely a consequence of differing prey availability during the two seasons which appeared to be influenced by environmental conditions and by stocking densities. Relative densities of rotifers, copepods, and cladocerans were monitored in conjunction with development of larvae in 1991. Results suggest that food availability limited the growth rates. Rearing alewife and blueback herring larvae in a continuous-flow system to a size capable of utilizing *Artemia* nauplii and subsequent transfer to aquaria for experimentation may enable controlled study of this life stage. Other factors that may contribute to successful rearing of these species in the laboratory are discussed.

INTRODUCTION

Controlled experimentation is a method to evaluate factors which affect the early life stages of fishes. However, laboratory culture of larvae of many types of fishes, especially marine species, is difficult and conditions necessary for successful culture remain unknown (Houde 1973; Kinne 1977). Many attempts to culture larvae of anadromous alewife (*Alosa pseudoharengus*) and blueback herring (*A. aestivalis*), collectively referred to as river herring, have been unsuccessful (Mansueti 1956; Norden 1967, 1968; Adams and Street 1969; Cianci 1969; Davis et al. 1972; Loesch and Kriete 1976; Lam and Roff 1977; Schubel et al. 1977; Kellogg 1982; Klauda and Palmer 1987). The principal complication to successful laboratory culture of these species appears to be an inability to induce larvae to actively feed. Heinrich (1981), however, reared alewife larvae to the juvenile stage in a closed system, feeding larvae with wild zooplankton supplemented with *Artemia* nauplii beginning 15 days after hatching.

Factors which limit the survival of fish larvae in culture include the availability of appropriate types of prey of suitable size and at suitable density (Houde 1973; Kinne 1977; Hunter 1984). Other factors also may influence the survival and growth of larvae in culture. Rearing container size may influence prey consumption and nutritional condition of larvae (Theilacker 1980; Barahona-Fernandes and Conan 1981). The size of the rearing container also may affect stimulation of larvae. Adequate stimulation by factors such as the direction, quantity, and quality of illumination as well as other visual, auditory, and olfactory stimuli may be vital for proper behavioral development of larval fish, including the development of proper feeding behavior (Blaxter 1969; Kinne 1977). Mortality not associated with nutritional condition may also affect larval survival. Blaxter (1968) suggested that pathogens, possibly associated with the accumulation of ciliates, may increase the mortality of aquaria-reared larvae.

The inability to rear alewife and blueback herring larvae has inhibited the acquisition of basic early life history information for these species. Studies of the tolerance of blueback herring larvae to temperature (Schubel et al. 1977) and to Ph and dissolved aluminum (Klauda and Palmer 1987), and of the tolerance of alewife and blueback herring larvae to suspended sediments (Auld and Schubel 1978) were limited to the prolarval stage due to high starvation-induced mortality of postlarvae. Kellogg (1982), evaluating thermal requirements for alewife larvae reared in fresh and oligohaline water up to 12 days after hatching, reported high, variable mortality which he suggested was associated with temperature-dependent utilization of endogenous energy reserves. A method for rearing and maintaining these species in the laboratory would enable experimental study of their postlarval biologies.

This report documents the culture of alewife and blueback herring larvae in a continuous-flow system. A continuous-flow system was designed because initial attempts to rear these larvae in aquaria were unsuccessful (Sismour and Loesch, in review). In this report, the growth of larvae cultured in the continuous-flow system, selected environmental conditions, and the potential zooplankton prey field are examined.

METHODS

Blueback herring prolarvae were reared in aquaria in 1989, but postlarvae failed to survive. A continuous-flow culture system was subsequently developed and was used to successfully rear postlarvae from fertilized eggs of unidentified river herring in 1990, and from artificially fertilized eggs of known alewife and blueback herring in 1991 (Sismour and Loesch, in review). Larvae of the 1991 alewife group were maintained in and sampled from both rearing tanks of the continuous-flow system prior to the transfer of blueback herring

prolarvae, but were sampled primarily from one tank. When blueback herring prolarvae were ready for transfer, alewife remaining in this tank were captured. The tank was drained and refilled, and blueback herring prolarvae were introduced. Alewife larvae were then subsampled from the second tank to complete the developmental series. The number of alewife larvae sampled from each rearing tank was not recorded. Once all alewife larvae were sampled, the second rearing tank was drained, refilled, and maintained without larvae to compare zooplankton relative densities between rearing tanks with and without larvae.

Larvae were subsampled throughout development, anaesthetized in tricaine methanosulfate (MS-222), and measured prior to fixation. Measurements were obtained using a Wild M3Z stereo-zoom microscope fitted with a calibrated micrometer; measurements were made to the nearest 0.5 micrometer unit. Prefixation measurements were analyzed to eliminate any effect of fixative-induced shrinkage. Mean length-at-age of larvae, instantaneous growth rate, and the instantaneous rate of decline in growth were estimated using the Gompertz growth equation of the FISHPARM computer program (Prager et al. 1987). Mean daily growth was estimated as the difference between mean length-at-age of sample i and mean length-at-age of sample $i+1$ divided by the intervening number of days.

Environmental data and zooplankton samples were obtained on most dates when larvae were sampled. Water temperatures in the Pamunkey River and in the rearing tanks were measured using a stem thermometer and water transparency was measured with a Secchi disc. Dissolved oxygen in the rearing tanks was determined by modified Winkler titration. Zooplankton were sampled from the culture tanks and from the river near the intake of the continuous-flow system. For each sample, zooplankton in 20 L of water pumped from a standard depth of 0.5 m was concentrated on a 35μ sieve and preserved in 5% PBF. In the laboratory, samples were adjusted to standard volume (110 ml). All rotifers

(*Keratella* spp. and "Rotifera spp."), copepods (nauplii and copepodites) and cladocerans were enumerated in four 1-mL aliquots, using a compound microscope at 40X magnification. Aliquots were obtained using a Stemple pipet and were dispensed into a Sedgewick-Rafter counting chamber for enumeration of zooplankton.

Alewife larvae were reared earlier during the 1991 season than were blueback herring larvae due to differing availability of adults. Alewife were reared from 12 April to 13 May and blueback herring were reared from 25 April to 6 June. Growth of the larvae differed during development and between the two groups. To compare the potential prey field during the development of each species, the 1991 study period was subdivided into three intervals: 12 April to 29 April (early), 30 April to 18 May (middle), and 19 May to 6 June (late). Relative densities of the zooplankton taxa in seven site-time groups were analyzed statistically using single classification fixed-effects ANOVA. Zooplankton relative densities were natural-logarithm transformed prior to analysis. *A priori* contrasts were used to test whether differences in zooplankton relative densities were significant between site-time groups during the culture of alewife and blueback herring larvae, between intervals of slower or faster growth within each species, and between slow-growth or fast-growth intervals between species. The Student-Newman-Kuels (SNK) test was used to determine which site-time groups differed significantly.

Single-classification fixed-effects ANOVA was used to test statistically the hypothesis that natural-logarithm transformed relative densities of zooplankton differed significantly between a rearing tank containing blueback herring larvae, a rearing tank without larvae, and the Pamunkey River. *A priori* contrasts were used to test whether differences between rearing tanks and between the river and the rearing tank without larvae were significant. The SNK test was used to determine which locations differed significantly.

RESULTS

Growth of alewife and blueback herring larvae

Table 1 summarizes the results of efforts to culture alewife and blueback herring larvae from 1989 to 1991. In 1989, groups of 25 larvae were transferred to 250-mL jars to test whether they would accept cultured prey organisms as food. Attempts to feed larvae with cultured zooplankton prey were unsuccessful, and larvae survived for about 8 days after hatching. The continuous-flow system was then developed to provide larvae with wild zooplankton prey drawn from within the historical nursery zone utilized by wild-spawned larvae of these species. Efforts to rear embryos from artificially fertilized eggs in 1990 using filtered (5.0μ) Pamunkey River water was unsuccessful due to extensive fungal infestation. In early May, 1990, naturally spawned eggs of unidentified river herring were collected from Herring Creek and were reared in water collected at the same location and filtered to 35μ. Using water from Herring Creek, fungal growth was not observed on eggs and hatching success was high (visually estimated to be greater than 90 percent). In 1991, alewife and blueback herring embryos were successfully reared in Herring Creek water, but eggs reared in water from Massaponax Creek (where ripe-running alewife were captured) were killed by fungal infestation.

The number of prolarvae used to stock rearing tanks in 1990 and 1991 was not standardized. No information was available to determine an optimal stocking density which was likely to vary depending on prey availability at the time of stocking. Minimum population size was estimated as the total number of larvae recovered for each group: a total of 51 larvae were recovered in 1990, and 280 alewife larvae and 521 blueback herring larvae were recovered in 1991 (Sismour and Loesch, in review). River herring larvae cultured in 1990 were reared in one of the two available tanks.

Postlarvae maintained in aquaria without food survived for about 8 days after hatching and grew only slightly, about 1.0 to 1.5 mm compared to about 2.2 to 5.8 mm for larvae transferred to rearing tanks with food (Table 2). Growth curves of larvae maintained in the rearing tanks differed between groups (Fig. 1). Postlarvae of the 1990 group were reared from a mean length of 4.6 mm at hatching to 19.6 mm for a single specimen sampled at 24 days after hatching. The growth curve for this group was approximately linear from 5 days to 16 days after hatching with a population growth rate of 0.8 mm d^{-1}. This high growth rate may be attributed to low population size (0.05 larvae L^{-1}) and presumably high zooplankton prey densities established in the rearing tank prior to introduction of larvae. In 1990, operation of the continuous-flow system began in mid-March, but larvae were not introduced until early May. This interval provided substantial time for development of high zooplankton densities, but samples were not taken to estimate potential prey abundance.

Larvae of the 1991 alewife group were reared from a mean length of 3.8 mm at hatching to a mean length of 17.3 mm at 32 days after hatching. Mean growth increased from 0.3 mm d^{-1} for postlarvae 4 days to 8 days after hatching to 0.6 mm d^{-1} for postlarvae 27 days to 32 days after hatching (Table 3). Instantaneous size-specific growth rate and instantaneous rate of decline in growth were 0.058 and 0.013, respectively. Blueback herring larvae were reared from a mean length of 3.9 mm at hatching to a mean length of 14.1 mm at 37 days after hatching. Mean growth decreased from 0.5 mm d^{-1} for postlarvae 5 days to 8 days after hatching to 0.1 mm d^{-1} for postlarvae 29 days to 37 days after hatching (Table 3). Instantaneous size-specific growth rate and instantaneous rate of decline in growth were 0.117 and 0.087, respectively.

The 1991 season was subdivided into three equal time intervals to facilitate analysis of growth in relation to zooplankton availability

and environmental conditions. The early interval, 10 April to 29 April, corresponds to the development of alewife larvae to 18 days after hatching; the middle interval, 30 April to 18 May, corresponds to development of alewife larvae after 18 days and of blueback herring larvae to 18 days; and, the late interval, 19 May to 6 June, corresponds to the development of blueback herring larvae after 18 days. Mean growth of both alewife and blueback herring larvae was higher during the middle interval, 0.6 mm d^{-1} and 0.4 mm d^{-1}, respectively, compared to 0.4 mm d^{-1} for alewife larvae during the early interval and 0.2 mm d^{-1} for blueback herring larvae during the late interval.

Water temperature and water transparency in the river and rearing tanks during the early, middle, and late time intervals are summarized in Table 4. Water temperature increased as the season progressed, but mean water temperatures in the river and rearing tanks did not differ significantly ($F=1.955$, $p=0.173$). Temperature in the rearing tanks was generally one to several degrees above the river (Fig. 2). Mean water temperature of the rearing tanks during the early time interval was significantly lower than during the middle interval ($t=4.920$, $p=0.000$), but did not differ significantly during the middle and late intervals ($t=2.022$, $p=0.058$). Water transparency varied as the season progressed (Fig. 3). Differences between the rearing tanks and the river were not significant ($F=3.975$, $p=0.056$) while differences between time intervals were significant ($F=8.847$, $p=0.001$). Mean transparency in the rearing tanks did not differ significantly ($t=0.006$, $p=0.995$) during the early and middle intervals, and was significantly lower during the late interval than during the middle interval ($t=2.883$, $p=0.011$). Dissolved oxygen, monitored only in the rearing tanks, consistently exceeded 7.0 mg L^{-1}.

Zooplankton availability

Mean relative densities and percent composition of rotifers, copepods (primarily nauplii), and cladocerans in the rearing tanks and Pamunkey River varied during development of river herring larvae (Table 5). The prey field was dominated by copepods and cladocerans early in the season, with rotifers becoming dominant during the middle interval; rotifers and copepods dominated the river zooplankton samples during the late interval (Fig. 4). The relative density of potential food particles (sum of the three zooplankton groups) in the river was low during the early interval, was highest during the middle interval, and declined to an intermediate level in the late interval (Fig. 4).

Mean relative densities ($x_i = \ln x_i$) of potential prey taxa differed significantly between site-time groups (Table 6). Mean relative densities of rotifers, copepods and cladocerans in the rearing tank containing alewife larvae and in the Pamunkey River did not differ significantly; whereas, mean relative densities of these groups were significantly lower in the rearing tank containing blueback herring compared to the river (Table 7). There appears to have been little or no effect from larval predation on zooplankton availability in the rearing tank containing alewife larvae. In situ reproduction or replenishment by zooplankton in entrained river water appears to have been sufficient to replace losses to predation. In comparison, blueback herring larvae appear to have caused mortality of zooplankton at a rate greater than could be sustained either by reproduction or replenishment.

Relative densities of rotifers and copepods in the rearing tank containing alewife larvae were significantly higher and the relative density of cladocerans was significantly lower during the fast-growth interval compared to the slow-growth intervals (Table 8). Similar results were observed between the slow-growth and fast-growth intervals during development of blueback herring larvae; relative densities of rotifers and copepods were significantly higher during the fast-growth

interval and differences in the relative densities of cladocerans were not significant (Table 8).

Comparing the slow-growth intervals, the mean relative density of rotifers was significantly lower and the mean relative density of cladocerans was significantly higher in the rearing tank containing alewife larvae compared to that containing blueback herring larvae. The mean relative densities of copepods between the two rearing tanks did not differ significantly (Table 9). Mean relative densities of potential prey taxa did not differ between rearing tanks containing alewife or blueback herring during the middle interval when growth of both species was highest (Table 9).

Table 10 illustrates the results of the SNK multiple comparison tests of zooplankton mean relative densities between site-time groups. Mean relative densities of rotifers during the middle interval and in the river during the later interval were significantly higher at all sites compared to the remaining groups, and were lowest during the early interval. Relative densities of copepods were significantly higher in the river during the middle and late periods and did not differ significantly between the rearing tanks, but were lower during the slow-growth periods of both groups of larvae compared to the fast-growth period. In contrast, mean relative density of cladocerans was significantly higher in the alewife rearing tank during the early period compared to the remaining site-time groups, and did not differ during the remaining intervals. Mean relative densities of cladocerans were slightly higher in river samples compared to samples from rearing tanks, although differences were not statistically significant.

Relative densities of zooplankton groups decreased time, both when herring larvae were included and excluded from rearing tanks (Table 11). The decline in both tanks suggests that environmental conditions may have contributed to reduced prey availability later in the season. Relative densities of the three prey groups differed significantly

between the two tanks and the Pamunkey River (Table 12). Relative densities for all three zooplankton groups were significantly higher in the tank without larvae than in the tank containing larvae (Table 13). In comparison, the relative densities of rotifers and copepods in the river and in the tank without larvae did not differ significantly, while differences in the relative densities of cladocerans were significant (Table 14). Mean relative densities of zooplankton were significantly lower in the rearing tank containing larvae compared either to the river or the rearing tank without larvae (Table 15).

Predation by blueback herring larvae appears to have significantly reduced the density of zooplankton in the rearing tank. Also, lower estimated relative densities of prey in this tank also might have been influenced by predator-induced behavioral responses not observed in the tank without larvae. Induced behavioral change in response to the presence of larval fish predators might have caused potential prey to remain near the bottom of the tank (Ringelberg 1991).

DISCUSSION

The greatest potential for mortality of larval fishes in a predator-free environment occurs at the time of transition to exogenous nourishment, and the likelihood of starvation-induced mortality decreases as larvae grow (Ehrlich 1974; Blaxter and Ehrlich 1974). In this study, food availability increased in synchrony with the nutritional need of developing alewife postlarvae enhancing growth, but lower prey availability earlier in the season may have caused relatively higher mortality at first-feeding. In comparison, the greater mean daily growth of blueback herring larvae during early development suggests adequate prey availability to facilitate first-feeding and survival of larvae. The resulting high abundance of larvae maintained the zooplankton population at low density which reduced larval herring growth with age. With increasing size, low prey density may have

promoted intra-specific competition for food, but this did not appear to be an important agent of mortality based on the number of blueback herring larvae recovered. Environmentally induced fluctuations in zooplankton survival or reproduction rates may have also contributed to lower prey availability later in the study period.

A superabundance of prolarvae of each species was transferred to the rearing tanks for the specific reason that conditions for survival of larvae were not known and, by stocking a surplus of larvae, the population size would adjust to the available density of prey through larval mortality. The lower estimated population size of alewife larvae compared to blueback herring larvae provides support for the conclusion that alewife larvae were food-limited at the time of first-feeding.

Supplying prey to alewife and blueback herring larvae that stimulates search and attack behavior and results in active feeding remains the greatest impediment to rearing alewife and blueback herring larvae in the laboratory. Heinrich (1981) successfully cultured alewife larvae in aquaria using wild zooplankton to feed larvae twice daily. However, other attempts to rear alewife and blueback herring larvae have been unsuccessful (see Introduction).

Copepod nauplii, cladocerans, and rotifers are the predominant components in diets of wild alewife and blueback herring larvae (Norden 1968; Heinrich 1981; Nigro and Ney 1982; Crecco and Blake 1983). These prey may provide sufficient nourishment to first-feeding larvae when presented in the correct proportion, density, and size range; however, the extent to which first-feeding river herring larvae might be reared on any one of these is not known. Copepod nauplii and small cladocera may provide sufficient nourishment for first-feeding river herring larvae, but they are difficult to culture in large quantities under controlled conditions (Houde 1973; Hunter 1984). Rotifers are relatively easier to culture (Theilacker and McMaster 1971; Hunter

1983), but intensive culture to feed large numbers of larvae daily is labor-intensive and requires the appropriate facilities and equipment.

Rotifers have been utilized successfully as a food for a variety of first-feeding clupeoid larvae. Hettler (1981) used the rotifer *Brachionus plicatilis* to rear first-feeding larvae of Atlantic menhaden (*Brevoortia tyrannus*). Although rotifers were not essential in rearing first-feeding Atlantic menhaden larvae, Hettler (1981) suggested that they may have provided a more reliable energy source. Growth rates of larval anchovy (*Engraulis mordax*) reared using *Brachionus plicatilis* exceeded the growth of a control group fed wild zooplankton (Theilacker and McMaster 1971).

The significance of rotifers in the diet of alewife and blueback herring larvae and the potential use of rotifers as a food for cultured larvae of these species remains questionable. Heinrich (1981) documented relatively low incidence of rotifers in the diet of cultured alewife. Norden (1968) reported an increase in the occurrence of rotifers in the guts of alewife larvae between 15 and 25 mm in length, but selection for rotifers was always negative. In comparison, Crecco and Blake (1983) reported positive selection for *Keratella* spp. and indicated that young larvae (5-12 mm) consumed mostly rotifers. Dietary preferences may differ between these species, and successful culture using rotifers for food may be more likely for blueback herring than for alewife. However, rotifers are an abundant component of the zooplankton community and apparent negative selection may not indicate the actual significance of rotifers in the diets of alewife or blueback herring larvae (Crecco and Blake 1983). Other factors, such as differential digestion, may also account for underestimation of contributions made by rotifers to the diets of river herring larvae (Gannon 1976). Rotifers may occur more frequently in guts of larger larvae compared to smaller larvae as the number of prey and their retention in the gut during

digestion increase. Evacuation of gut contents during capture and fixation may also bias the estimated composition of consumed prey.

Artemia (brine shrimp) nauplii are easily cultured and have been used extensively as prey for fish larvae (Hunter 1983), but this food is too large for larvae of many species of smaller marine fishes to consume at first-feeding (Houde 1973). For clupeid species with smaller larvae, *Artemia* nauplii have been introduced subsequent to first-feeding after attaining suitable size. Heinrich (1981) reared first-feeding alewife larvae using wild zooplankton and introduced *Artemia* nauplii into the diet beginning 15 days after hatching. *Artemia* nauplii and powdered trout food were introduced to Atlantic menhaden larvae at lengths of 10 to 12 mm (Hettler 1981). Houde and Palko (1970) reared first-feeding larvae of the scaled herring, *Harengula pensacolae*, on wild zooplankton, introduced *Artemia* nauplii on the 12th day after hatching, and indicated that juveniles greater than 35 mm consumed pelleted food. Winston (1988) supplemented the diet of cultured gizzard shad with *Artemia* nauplii beginning at 18 days after hatching.

Other factors may also facilitate survival of larvae in aquaria. Atlantic menhaden larvae (Hettler 1981) and scaled sardine larvae (Houde and Palko 1970) were reared in water containing a bloom of the green alga *Chlorella* spp. which has been suggested to "condition" water by removing metabolic wastes produced by fish larvae and zooplankton (Houde 1973). *Chlorella* may also supplement the diet of some species of larval fishes directly through incidental consumption and digestion, or indirectly from algal cells present in the gut of ingested zooplankton (Moffatt 1981). However, its significance as a dietary supplement for larval fish, in general, has not been established conclusively (Houde 1973). Nematipour, Nakagawa, and Ohya (1990) reported increased mobilization of lipid reserves in juvenile ayu (*Pleccoglossus altivelis*) fed a commercial diet supplemented with 1 percent *Chlorella* extract compared to those fed the commercial diet without supplementation.

Consumption and digestion of *Chlorella* by larval fishes might enhance the availability of body lipids thereby delaying the onset of starvation and prolonging the time available to find suitable food; however, this remains to be demonstrated experimentally and may be of little significance to larvae prior to accumulation of body lipids (Ehrlich 1974).

Various factors influence the survival and growth potential of fish larvae reared in the laboratory. Further elaboration of the environmental and prey requirements of alewife and blueback herring larvae are necessary before routine laboratory culture of these taxa becomes possible. Heinrich (1981) demonstrated that rearing alewife in the laboratory is technically feasible; however, an alternative to multiple daily collection of zooplankton is desirable, especially in those circumstances where daily maintenance of the larvae may not be possible. By using a continuous-flow system such as described by Sismour and Loesch (in review), it is possible to rear alewife and blueback herring postlarvae to a size capable of consuming *Artemia* nauplii and it eliminates the need for daily collection of wild zooplankton prey. It also may provide a basis for developing a system for rearing these species under more controlled conditions.

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LITERATURE CITED

Adams, J. G. and M. W. Street. 1969. Notes on the spawning and embryological development of blueback herring (*Alosa aestivalis* Mitchill) in the Altamaha River, Georgia. Georgia Game and Fish Commission, Marine Fisheries Division, Contribution Series 16, Brunswick, Georgia.

Auld, A. H. and J. R. Schubel. 1978. Effects of suspended sediment of fish eggs and larvae: A laboratory assessment. Estuarine and Coastal Marine Science 6: 153-164.

Barahona-Fernandes, M. H. and G. Conan. 1981. Daily food intake of reared larvae of the European seabass (*Dicentrarchus labrax* L.) statistical analysis and modelling. Rapports et Proces-Verbaux des Reunions, Consel International pur l'Exploration de la Mer 178: 41-44.

Blaxter, J. H. S. 1968. Rearing herring larvae to metamorphosis and beyond. Journal of the marine biological Association, United Kingdom 48: 17-28.

Blaxter, J. H. S. 1969. Development: eggs and larvae, pages 177-252. *In*: W.S. Hoar and D.J. Randall, eds. Fish physiology. Academic Press, New York. 485 p.

Blaxter, J. H. S. and K. F. Ehrlich. 1974. Changes in behavior during starvation of herring and plaice larvae, pages 575-588. *In*: J.H.S. Blaxter, ed. The early life history of fish. Springer-Verlag, Berlin.

Cianci, J. M. 1969. Larval development of the alewife, *Alosa pseudoharengus* Wilson, and the glut herring, *Alosa aestivalis* Mitchill. M.S. thesis, University of Connecticut, Storrs, Connecticut., U.S.A. 62 p.

Crecco, V. and M. M. Blake. 1983. Feeding ecology of coexisting larvae of American shad and blueback herring in the Connecticut River. Transactions of the American Fisheries Society 112: 498-507.

Davis, J., J. V. Merriner, W. J. Hoagman, R. A. St. Pierre and W. L. Wilson. 1972. Biology and utilization of anadromous alosids; Job 6: Development of culture technique. Annual Progress Report, AFC 7-2, United States Bureau for Commercial Fisheries.

Ehrlich, K. F. 1974. Chemical changes during growth and starvation of herring larvae, pages 301-324. *In*: J. H. S. Blaxter (editor), The early life history of fish. Springer-Verlag, Berlin.

Gannon, J. E. 1976. The effects of differential digestion rates of zooplankton by alewife, *Alosa pseudoharengus*, on determination of selective feeding. Transactions of the American Fisheries Society 105: 89-95.

Heinrich, J. W. 1981. Culture, feeding, and growth of alewifes hatched in the laboratory. Progressive Fish-Culturist 43: 3-7.

Hettler, W. F. 1981. Spawning and rearing Atlantic menhaden. Progressive Fish-Culturist 43: 80-84.

Houde, E. D. 1973. Some recent advances and unsolved problems in the culture of marine fish larvae. Proceedings of the World Mariculturist Society. 3: 83-112.

Houde, E. D. and B. J. Palko. 1970. Laboratory rearing of the clupeid fish *Harengula pensacolae* from fertilized eggs. Marine Biology 5: 354-358.

Hunter, J. R. 1984. Synopsis of culture methods of marine fish larvae, pages 24-27. *In*: H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, W. W. Kendall, Jr. and S. L. Richardson (editors), Ontogeny and systematics of fishes. Special Publication Number 1, American Society of Ichthyologists and Herpetologists, Allen Press, Inc., Lawrence, Kansas. 760 p.

Kellogg, R. L. 1982. Temperature requirements for the survival and early development of the anadromous alewife. Progressive Fish-Culturist 44: 63-73.

Kinne, O. 1977. Cultivation of animals: research cultivation - Pisces, pages 968-1035. *In*: O. Kinne (editor), Marine Biology: A comprehensive, integrated treatise on life in oceans and coastal waters, Volume III, part 2. John Wiley and Sons, Chichester.

Klauda, R. J. and R. E. Palmer. 1987. Responses of blueback herring eggs and larvae to pulses of acid and aluminum. Transactions of the American Fisheries Society 116: 561-569.

Lam, C. N. H. and J. C. Roff. 1977. A method for separating alewife *Alosa pseudoharengus* from gizzard shad *Dorosoma cepedianum* larvae. Journal of Great Lakes Research 3: 313-316.

Loesch, J. G. and W. H. Kriete, Jr. 1976. Biology and management of river herring and shad in Virginia. Completion Report, 1974-1976. National Marine Fisheries Service, Project Number AFC 8-1 to 8-3.

Mansueti, R. J. 1956. Alewife herring eggs and larvae reared successfully in lab. Maryland Tidewater News 13: 2-3.

Moffatt, N. M. 1981. Survival and growth of Northern Anchovy larvae on low zooplankton densities as affected by the presence of a *Chlorella* bloom. Rapports et Proces-Verbaux des Reunions, Consel International pur l'Exploration de la Mer 178: 475-480.

Nematipour, G. R., H. Nakagawa and S. Ohya. 1990. Effect of *Chlorella*-extract supplementation to diet on in vitro lipolysis in ayu. Nippon Suisan Gakkaishi 56: 777-782.

Nigro, A. A. and J. J. Ney. 1982. Reproduction and early life history accommodations of landlocked alewives to a southern range extension. Transactions of the American Fisheries Society 111: 559-569.

Norden, C. R. 1967. Development and identification of the larval alewife, *Alosa pseudoharengus* (Wilson), in Lake Michigan. Journal of Great Lakes Research 10: 70-78.

Norden, C. R. 1968. Morphology and food habits of the larval alewife, *Alosa pseudoharengus* (Wilson), in Lake Michigan. Journal of Great Lakes Research 11: 103-110.

Prager, M. H., S. B. Saila, and C. W. Recksiek. 1987. FISHPARM: A microcomputer program for parameter estimation of nonlinear models in fishery science. Technical Report. 87-10, Old Dominion University Research Foundation.

Ringelberg, J. 1991. A mechanism of predator-mediated induction of diel vertical migration in *Daphnia hyalina*. Journal of Plankton Research 13: 83-89.

Schubel, J. R., T. S. Y. Koo, and C. F. Smith. 1977. Thermal effects of power plant entrainment on survival of fish eggs and larvae: A laboratory assessment. Chesapeake Science 18: 290-298.

Theilacker, G. H. 1980. Rearing container size affects morphology and nutritional condition of larval jack mackerel, *Trachurus symmetricus*. Fishery Bulletin, U.S. 78: 789-791.

Theilacker, G. H. and M. F. McMaster. 1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Marine Biology. 10: 183-188.

Winston, M. R. 1988. Culturing larval gizzard shad in laboratory aquaria. Progressive Fish-Culturist. 50: 118-119.

Table 1. Summary of the culture of alewife and blueback herring larvae, 1989-1991.

Year	Species	Source of adults	Water source	Mean Water temperature	Water treatment	Results
1989	Blueback	Pamunkey River	Pamunkey R. (mile 39)	20 C	Filtered (0.5μ)	Successful hatch.
1990	Alewife	Massaponax Creek	Pamunkey R. (mile 17)	15 C	Filtered (5.0μ)	Fungal growth, poor hatch, system failure.
	Alewife	Massaponax Creek	Pamunkey R. (mile 17)	NR	Filtered (5.0μ)	Fungal growth, unsuccessful hatch.
	Blueback	Pamunkey River	Pamunkey R. (mile 17)	NR	Filtered (5.0μ), fungal inhibitor	Fungal growth, unsuccessful hatch.
	Unid. River herring	Herring Creek	Herring Creek	NR	Filtered (35μ), fungal inhibitor	Successful hatch.
1991	Alewife	Massaponax Creek	Herring Creek	21 C	Filtered (35μ)	Successful hatch.
	Alewife	Massaponax Creek	Massaponax Creek	18 C	Filtered (35μ)	Fungal growth, poor hatch.
	Blueback	Herring Creek	Herring Creek	21 C	Filtered (35μ)	Successful hatch.

Table 2. Mean length-at-age (in millimeters) of river herring prolarvae and postlarvae (fed and starved) during the spring spawning seasons in 1989, 1990, and 1991.

Age (days)	Fed larvae			Starved larvae		
	1990 River herring	1991 Alewife	1991 Blueback herring	1989 Blueback herring	1991 Alewife	1991 Blueback herring
			Prolarvae			
0	4.6	3.5	4.0	4.0	3.5	4.0
1	5.3	4.3				
2			5.1	5.1		5.1
			Postlarvae			
3						
4		5.1		5.4	5.1	
5	7.8					5.0
6				5.5		
7						
8	10.4	5.8	7.0	5.3	5.0	

Table 3. Estimated mean length-at-age and mean daily growth increment of cultured alewife and blueback herring postlarvae.

Age (days)	Mean length (mm)	Daily growth increment (mm)	Age (days)	Mean length (mm)	Daily growth increment (mm)
	Alewife			**Blueback herring**	
0	3.8		0	3.9	
1	4.0	0.2	2	4.8	0.5
4	4.7	0.2	5	6.3	0.5
8	5.9	0.3	8	7.6	0.5
12	7.2	0.3	13	9.7	0.4
14	7.9	0.4	17	11.0	0.3
18	9.6	0.4	21	12.0	0.3
21	11.0	0.5	25	12.8	0.2
24	12.5	0.5	29	13.4	0.2
27	14.2	0.6	34	13.9	0.1
32	17.3	0.6	37	14.2	0.1

Table 4. Mean water temperature and mean water transparency of the Pamunkey River and of the rearing tanks during culture of alewife and blueback herring larvae in 1991.

Location	Time period	Water temperature		Water transparency	
		Mean	S.D.	Mean	S.D.
Pamunkey River	10 Apr - 29 Apr	17.7	1.3	50.3	8.5
	30 Apr - 18 May	22.9	2.5	56.0	5.5
	19 May - 6 Jun	26.5	2.4	48.0	4.5
Rearing Tanks	10 Apr - 29 Apr	17.6	3.2	57.8	7.0
	30 Apr - 18 May	24.6	2.5	57.8	6.9
	19 May - 6 Jun	28.0	2.4	46.6	2.3

Table 5. Mean (geometric) relative density and percent composition (in parentheses) of zooplankton potential prey (L^{-1}) in the Pamunkey River and in rearing tanks during the culture of alewife and blueback herring larvae.

Zooplankton taxa	Time Interval		
	10 Apr - 29 Apr	30 Apr - 18 May	19 May - 6 June
	Pamunkey River		
Rotifera	26 (32.0)	384 (80.7)	344 (65.3)
Copepoda	11 (13.6)	74 (15.5)	158 (30.0)
Cladocera	44 (54.3)	18 (3.8)	25 (4.7)
	Alewife rearing tank		
Rotifera	74 (56.5)	477 (89.3)	
Copepoda	17 (13.0)	48 (9.0)	
Cladocera	40 (30.5)	9 (1.7)	
	Blueback herring rearing tank		
Rotifera		356 (87.9)	98 (73.1)
Copepoda		38 (9.4)	25 (18.7)
Cladocera		11 (2.7)	11 (8.2)

Table 6. Analysis of variance summary tables for relative densities of zooplankton groups between site-time groups during the culture of alewife and blueback herring larvae in 1991.

Source	d.f.	SS	MS	*F*	P
		Rotifera			
Between groups	6	253.71	42.29	46.46	<0.001
Within groups	121	110.12	0.91		
Total	127	363.83			
		Copepoda			
Between groups	6	72.94	12.16	23.51	<0.001
Within groups	121	62.57	0.52		
Total	127	135.50			
		Cladocera			
Between groups	6	72.35	12.06	9.19	<0.001
Within groups	121	158.70	1.31		
Total	127	231.04			

Table 7. A priori comparisons of mean relative densities (x_l= ln x_i) of zooplankton (L^{-1}) in the Pamunkey River and in rearing tanks containing alewife and blueback herring larvae.

Prey Taxon	Location	Mean (geometric)	L	Standard Error	t	P
		Alewife				
Rotifera	Pamunkey R.	4.2	-0.897	0.539	-1.665	0.101
	Rearing Tank	4.7				
Copepoda	Pamunkey R.	3.6	0.433	0.375	1.157	0.253
	Rearing Tank	3.4				
Cladocera	Pamunkey R.	3.1	-0.917	0.606	-1.512	0.139
	Rearing Tank	3.6				
		Blueback herring				
Rotifera	Pamunkey R.	5.9	1.332	0.294	4.529	0.000
	Rearing Tank	5.2				
Copepoda	Pamunkey R.	4.8	2.634	0.356	7.401	0.000
	Rearing Tank	3.4				
Cladocera	Pamunkey R.	3.0	1.351	0.630	2.144	0.037
	Rearing Tank	2.3				

Table 8. A priori comparisons of mean relative densities (x_i= ln x_i) of zooplankton (L^{-1}) in rearing tanks during intervals of slow- and fast-growth by alewife and blueback herring larvae.

Prey Taxon	Interval	Mean (geometric)	L	Standard Error	t	P
		Alewife				
Rotifera	Slow growth	3.2	-2.899	0.402	-7.215	0.000
	Fast growth	6.1				
Copepoda	Slow growth	3.1	-0.571	0.245	-2.328	0.026
	Fast growth	3.7				
Cladocera	Slow growth	4.6	2.019	0.382	5.289	0.000
	Fast growth	2.6				
		Blueback Herring				
Rotifera	Slow growth	4.6	1.296	0.148	8.744	0.000
	Fast growth	5.9				
Copepoda	Slow growth	3.2	0.393	0.154	2.550	0.016
	Fast growth	3.6				
Cladocera	Slow growth	2.4	0.074	0.329	0.225	0.824
	Fast growth	2.3				

Table 9. A priori comparisons of mean relative densities (x_i= ln x_i) of zooplankton (L^{-1}) in rearing tanks containing alewife and blueback herring larvae during slow- and fast-growth intervals.

Prey Taxon	Species	Mean (geometric)	L	Standard Error	t	P
		Slow-growth				
Rotifera	Alewife	3.2	-1.391	0.392	-3.552	0.002
	Blueback	4.6				
Copepoda	Alewife	3.1	-0.118	0.235	-0.502	0.619
	Blueback	3.2				
Cladocera	Alewife	4.6	2.278	0.238	9.593	0.000
	Blueback	2.3				
		Fast-growth				
Rotifera	Alewife	6.1	0.212	0.174	1.219	0.237
	Blueback	5.9				
Copepoda	Alewife	3.7	0.060	0.169	0.355	0.726
	Blueback	3.6				
Cladocera	Alewife	2.6	0.186	0.444	0.418	0.679
	Blueback	2.4				

Table 10. Multiple comparison tests (Student-Newman-Kuels procedure) of mean relative densities ($x_i = \ln x_i$) of zooplankton (L^{-1}) between site-time groups during the culture of alewife and blueback herring larvae. Sampling locations and time intervals are indicated by the following abbreviations: A) alewife culture tank; B) blueback herring culture tank; P) Pamunkey River; e) early, 10 April - 29 April, time interval; m) middle, 30 April - 18 May, time interval; and 1) late, 19 May - 6 June, time interval. Bars indicate homogeneous subsets (95% CI).

Rotifera							
Site-time group	P,e	A,e	B,l	P,l	B,m	P,m	A,m
Mean relative density	2.4	3.2	4.6	5.8	5.9	6.0	6.1
	—						
		—					
			—				
				—	—	—	—
Copepoda							
Site-time group	P,e	A,e	B,l	B,m	A,m	P,m	P,l
Mean relative density	2.9	3.1	3.2	3.6	3.7	4.4	5.1
	—	—	—				
		—	—	—	—		
						—	—
Cladocera							
Site-time group	P,e	A,e	B,l	B,m	A,m	P,m	P,l
Mean relative density	2.3	2.4	2.6	2.8	3.2	3.4	4.6
	—	—	—	—	—		
		—	—	—	—	—	
							—

Table 11. Mean (geometric) relative density and percent composition (in parentheses) of zooplankton potential prey (L^{-1}) in rearing tanks with and without blueback herring larvae.

Taxon	Sampling Dates 17-21 May	25-29 May	3-6 June	Mean
		With larvae		
Rotifera	257 (88.0)	110 (73.3)	60 (62.5)	142
Copepoda	26 (9.0)	27 (18.0)	29 (30.2)	27
Cladocera	9 (3.0)	13 (8.7)	7 (7.3)	10
		Without larvae		
Rotifera	854 (73.5)	347 (46.1)	245 (52.2)	482
Copepoda	245 (21.1)	257 (34.2)	172 (36.7)	225
Cladocera	63 (5.4)	148 (19.7)	52 (11.1)	88

Table 12. Analysis of variance summary tables for relative densities of zooplankton groups sampled from a rearing tanks with and without blueback herring larvae and in the Pamunkey River near the culture system intake.

Source	d.f.	SS	MS	F	P
		Rotifera			
Between groups	2	24.585	12.292	31.60	<0.001
Within groups	69	26.845	0.389		
Total	71	51.430			
		Copepoda			
Between groups	2	66.045	33.022	92.62	<0.001
Within groups	69	24.601	0.357		
Total	71	90.646			
		Cladocera			
Between groups	2	57.706	28.853	28.39	<0.001
Within groups	69	70.121	1.016		
Total	71	127.827			

Table 13. A priori comparisons of the mean relative densities (x_i= ln x_i) of zooplankton (L^{-1}) in rearing tanks with and without blueback herring postlarvae.

Prey Taxon	Rearing Tank	Mean (geometric)	L	Standard Error	t	P
Rotifera	with larvae	4.8	-1.242	0.180	-6.895	0.000
	without larvae	6.0				
Copepoda	with larvae	3.3	-2.100	0.125	-16.813	0.000
	without larvae	5.4				
Cladocera	with larvae	2.1	-2.193	0.255	8.617	0.000
	without larvae	4.3				

Table 14. A priori comparisons of the mean relative densities (x_i= ln x_i) of zooplankton (L^{-1}) in the Pamunkey River and in the rearing tank without blueback herring postlarvae.

Prey Taxon	Location	Mean (geometric)	L	Standard Error	t	P
Rotifera	Pamunkey R.	6.0	-0.004	0.180	-0.021	0.983
	Rearing Tank	6.0				
Copepoda	Pamunkey R.	5.3	-0.145	0.180	-0.807	0.426
	Rearing Tank	5.4				
Cladocera	Pamunkey R.	3.2	-1.109	0.283	-3.919	0.000
	Rearing Tank	4.3				

Table 15. Multiple comparison tests (Student-Newman-Kuels procedure) of mean relative densities ($x_i = \ln x_i$) of zooplankton (L^{-1}) between the Pamunkey River and rearing tanks with and without blueback herring larvae. Sampling locations are indicated by the following abbreviations: PR) Pamunkey River; WB) with blueback herring; and NB) without blueback herring. Bars indicate homogeneous subsets (95% CI).

Rotifera			
Sampling location	WB	PR	NB
Mean relative density	4.8	6.0	6.0
	—		
		—	—

Copepoda			
Sampling location	WB	PR	NB
Mean relative density	3.3	5.3	5.4
	—		
		—	—

Cladocera			
Sampling location	WB	PR	NB
Mean relative density	2.1	3.1	4.3
	—		
		—	
			—

Figure 1. Growth curves of alewife and blueback herring larvae reared in 1990 and in 1991. Growth curves are based on mean length-at-age (mm) estimated by fitting observed length-at-age to the Gompertz equation. The final value for the 1990 river herring group represents a single specimen.

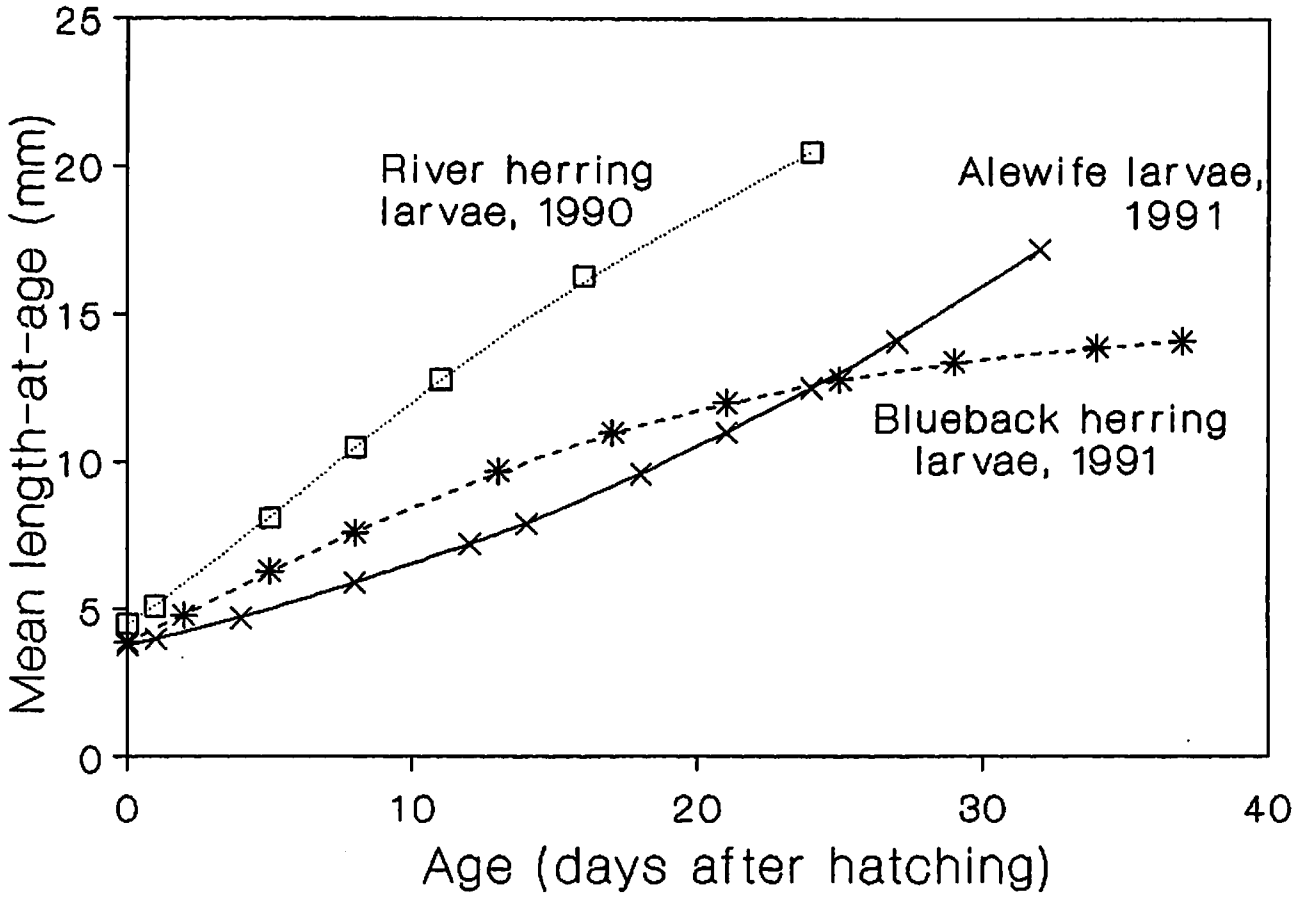

25
20
15
10
5
0
Mean length-at-age (mm)
River herring
larvae, 1990
Alewife larvae,
1991
Blueback herring
larvae, 1991
0
10
20
30
40
Age (days after hatching)

Figure 2. Water temperature measured in the Pamunkey river (solid line) and in rearing tanks (dashed line) during the culture of alewife larvae and blueback herring larvae in 1991.

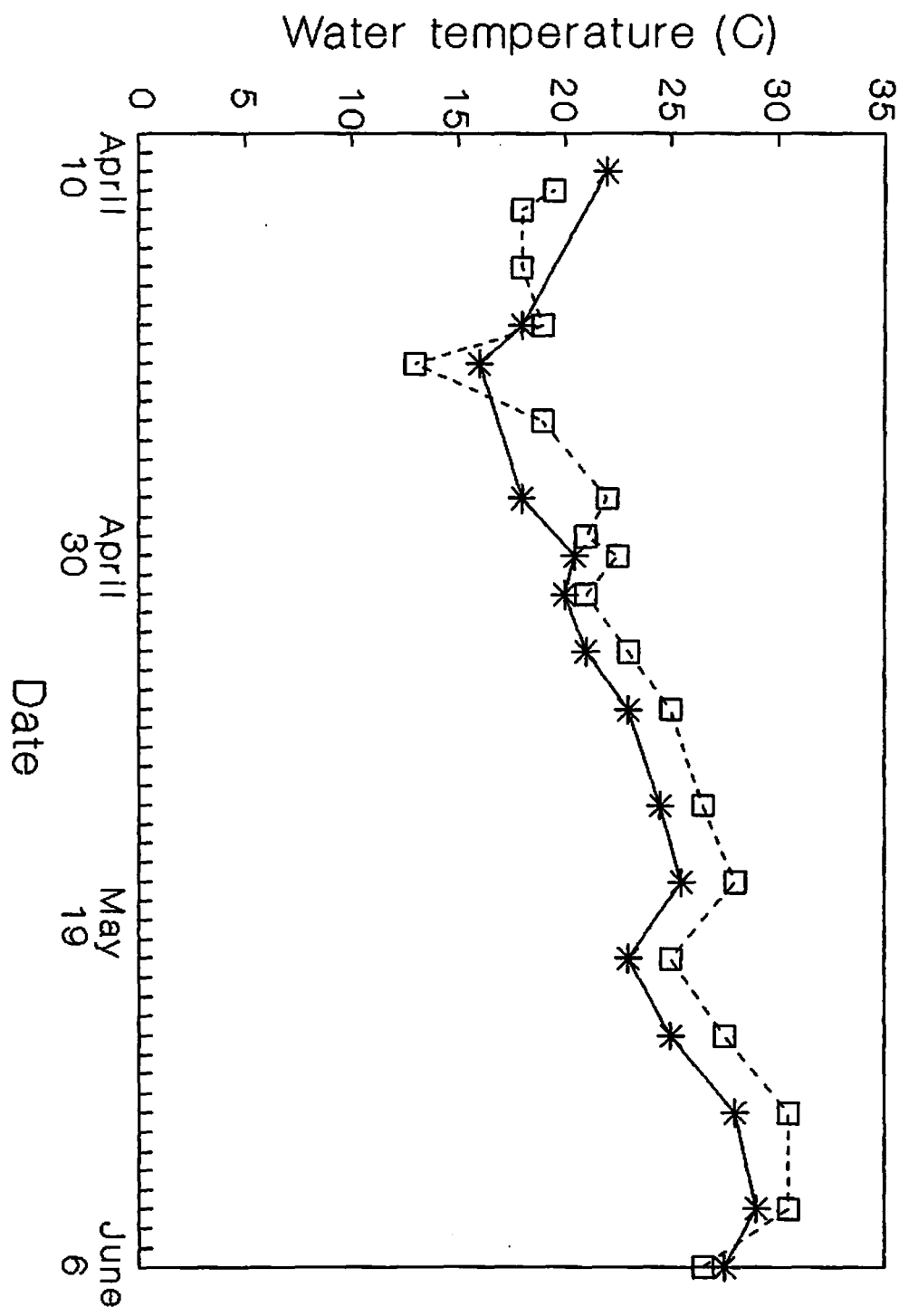

Water temperature (C)
0
5
10
15
20
25
30
35
April
10
April
30
May
19
June
6
Date

Figure 3. Water transparency measured using a Secchi disc in the Pamunkey river (solid line) and in rearing tanks (dashed line) during the culture of alewife larvae and blueback herring larvae in 1991.

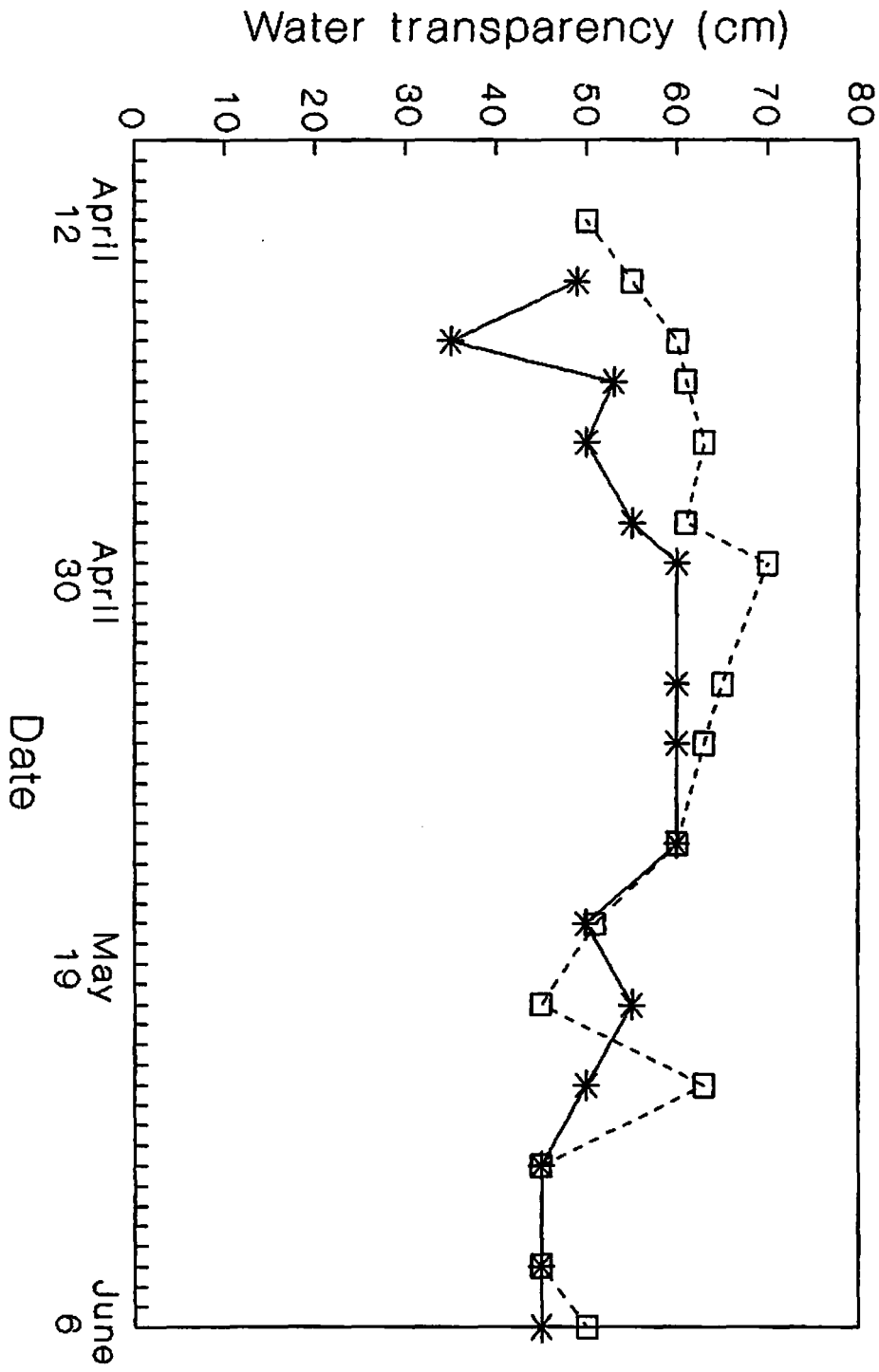

Water transparency (cm)
0
10
20
30
40
50
60
70
80
April 12
April 30
May 19
June 6
Date

Figure 4. Relative density and percent composition of zooplankton sampled from the Pamunkey River adjacent to the culture system intake during the 1991 season that are potential prey for alewife and blueback herring larvae. The upper panel presents the sum of the mean relative densities of each group by date and the lower panel presents the percent composition of each group in the total.

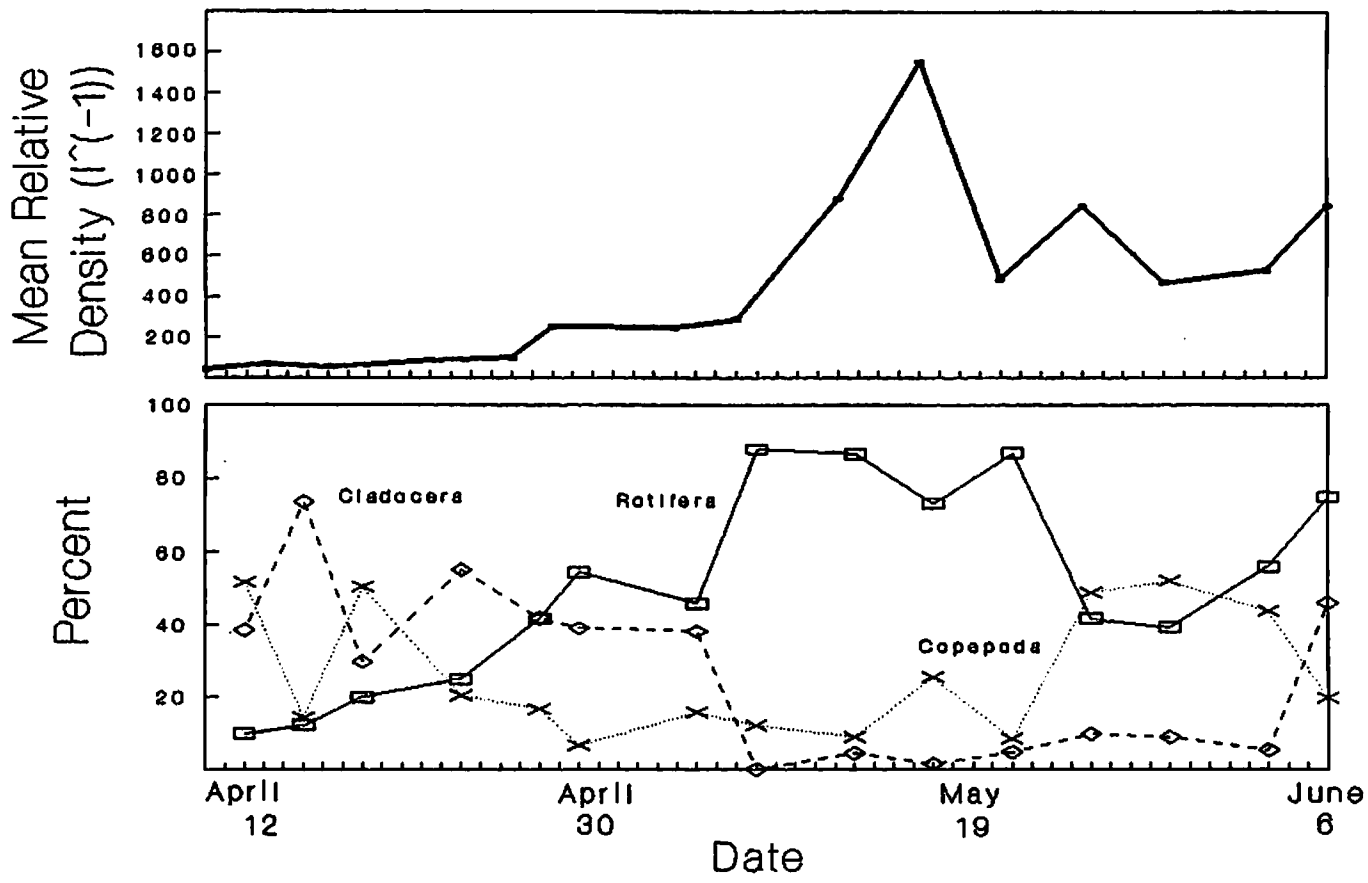
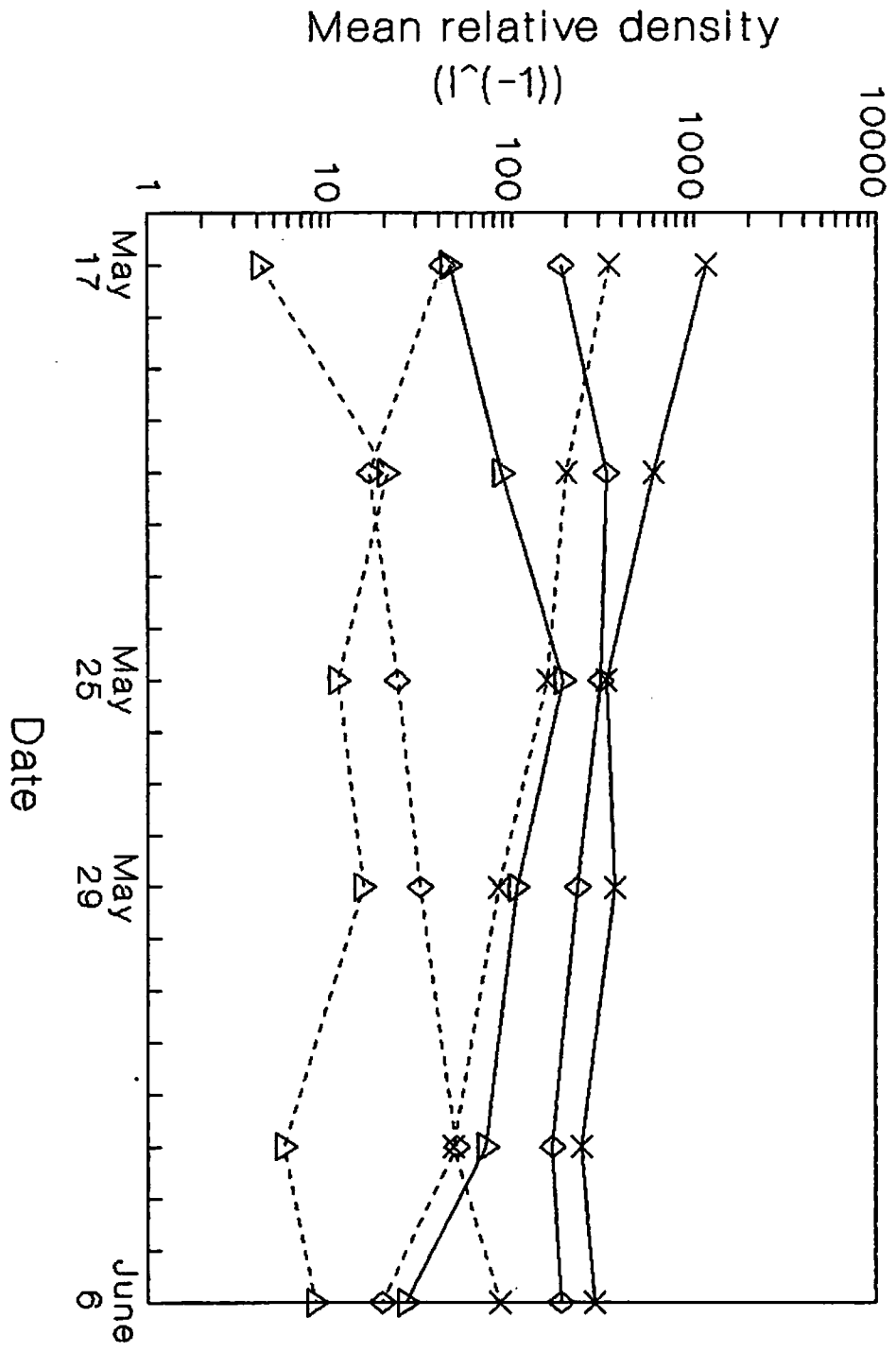

Mean Relative Density (l^(-1))
1600
1400
1200
1000
800
600
400
200
Percent
100
80
60
40
20
Cladocera
Rotifera
Copepoda
April 12
April 30
May 19
June 6
Date

Figure 5. Mean relative densities of rotifera (×), copepoda (◊), and cladocera (▵) in rearing tanks with blueback herring larvae (dashed line) and without blueback herring larvae (solid line) during the 1991 study period.


Mean relative density
(l^(-1))
1
10
100
1000
10000
May
17
May
25
May
29
June
6
Date

APPENDIX C.

Characteristics of Alewife (*Alosa pseudoharengus*) and Blueback Herring (*A. aestivalis*) Early Life Stages with Emphasis on the Identification of Postlarvae

Characteristics of Alewife (*Alosa pseudoharengus*) and Blueback Herring (*A. aestivalis*) Early Life Stages With Emphasis on the Identification of Postlarvae

by

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1. This study was conducted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Previous studies have not resolved the problem of taxonomic delineation of alewife and blueback herring larvae. Morphometric, meristic, and pigmentation characteristics of alewife and blueback herring larvae reared from eggs of known parentage were examined to identify characteristics useful for taxonomic delineation. Alewife and blueback herring larvae were reared in a continuous flow system for 32 days and for 37 days, respectively. Morphological measurements were obtained from anesthetized larvae, and meristic and pigment characters were examined using fixed and preserved specimens. The results indicate that morphometric and meristic characters are inadequate to delimit larvae of these species, but pigment characters of taxonomic value were identified. Among preflexion larvae, pigment at the dorsal margin of caudal-region myomeres and the fin-fold was more common for alewife than blueback herring, but was not diagnostic. Blueback herring postflexion larvae exhibited heavier pigmentation, especially along the mid-line posterior to the dorsal fin, and larvae larger than 11 mm SL were characterized by pigment on the dorsal surface of the notochord at the level of the nape. In comparison, alewife larger than 15 mm SL were characterized by melanophores along the lateral aspect of the notochord in the same region. Species-specific distributions of xanthochrome were observed in anesthetized specimens, but were lost from fixed and preserved specimens. Pigment characters are suggested to delimit alewife and blueback herring postflexion larvae, but other methods appear necessary to delimit preflexion larvae.

INTRODUCTION

Accurate identification of eggs and larvae is an essential component of early life history studies of fishes. As Powles and Markle (1983) state: "Minor errors in identification of larval fishes can lead to major misinterpretations of ecological and taxonomic phenomena." Alewife and blueback herring eggs and larvae may be intermixed on spawning and nursery grounds, such as in the Chesapeake Bay (Dovel 1971), the Delaware River (Wang and Kernehan 1979), and the Hudson River (Boreman 1981). Identification of the early life stages of these species is necessary to understand the ecology of these larvae in areas of potential sympatry.

Total myomere or vertebral number has been suggested to be the most useful taxonomic character for classifying larval fishes (Berry and Richards 1973; McGowan and Berry 1983). Berry and Richards (1973) suggest that total vertebral or total myomere numbers can be useful in the separation of larval clupeids. However, Russell (1976) indicates that obtaining counts of total myomeres for preflexion clupeid larvae can be difficult due to indistinct myomere separation anterior to the cleithrum or posterior to the vent. The utility of vertebral or myomere counts for classifying larvae of closely related clupeid species appears to vary. Bulak (1985) found no difference in total vertebral number or total myomere number for blueback herring (*Alosa aestivalis*) and gizzard shad (*Dorosoma cepedianum*) in the Santee-Cooper River, South Carolina, but demonstrated significantly lower total vertebral and total myomere numbers for threadfin shad (*D. petenense*) compared to gizzard shad. In contrast, Taber (1969 cited by Lam and Roff 1977) found no difference between threadfin shad and gizzard shad in Lake Texoma, Oklahoma.

Differences in morphometric and meristic characters of many clupeoid larvae often appear to be of greater value for classifying specimens to a genus than to a species (Houde and Fore 1973; Richards et al. 1974; Funes-Rodriguez and Esquivel-Herrera 1985), and classification

of larvae may be simplified once the complete complement of dorsal and anal fin rays and gillrakers have formed in older larvae (Wang 1970; McGowan and Berry 1983; Bulak 1985). Other information, such as time of year or location of capture, may be essential to confirm the identity of larvae, but positive identification based on meristics and morphometrics may not be possible for sympatric congeners (Houde and Fore 1973; Richards et al. 1974; Taber 1969 cited in Lam and Roff 1977; Funes-Rodriguez and Esquivel-Herrera 1985).

Prior taxonomic studies of alewife and blueback herring larvae have produced conflicting results. Chambers et al. (1976) suggested that alewife and blueback herring larvae may be delimited on the basis of meristic and morphometric characters. In contrast, Cianci (1969) found no significant differences in morphometric and meristic characters between larvae of these species reared from eggs. Related studies do not support the conclusion of significant meristic and morphometric variation between alewife and blueback herring larvae (Lam and Roff 1976; Bulak 1985). Following transformation, juveniles may be delimited based on gill raker morphology (Wang 1970), pigment patterns on the tongue (Dovel et al. 1965), and by peritoneal pigmentation (Wang 1970; Loesch 1987).

Wang (1970) described pigmentation characteristics for postlarval and juvenile alewife (15+ mm SL) and juvenile blueback herring (20+ mm SL). Alewife from 15 to 20 mm SL were characterized by two rows on the dorsum from the back of the head to caudal peduncle and by melanophores on the epaxial portion of the body, and larvae from 20 to 25 mm SL were characterized by dense melanophores on the epaxial portion of the body and a dark lateral band above the base of the epaxial portion of the body. In comparison, blueback herring from 20 to 23 mm SL were characterized by two rows of melanophores between the dorsal fin and the caudal peduncle with no melanophores on the epaxial surface of the body, and larvae greater than 23 mm SL were characterized by melanophores

along the dorsum extending to the head and with scattered melanophores on the epaxial portion of the body.

Larval pigmentation may provide information for establishing taxonomic identity at the species level (Berry and Richards 1973; Ahlstrom and Moser 1976; McGowan and Berry 1983). Differences may exist in the general distribution and developmental timing of certain pigmentation characteristics, and diagnostic pigmentation characters may be present. Pigmentation characters of potential taxonomic value for delimiting alewife and blueback herring larvae have not been examined. The presence of melanophores at the base of the fin-fold at the dorsal margin of myomeres posterior to the vent has been reported to be present in alewife by Norden (1967) and Cianci (1969) but was not reported in alewife by Mansueti (1956). The occurrence of this pigmentation was not observed in blueback herring larvae by Cianci (1969), and it was not noted by Kuntz and Radcliffe (1917) although it was displayed in their illustration of a blueback herring larva (Fig. 99, Kuntz and Radcliffe 1917).

The present investigation examines alewife and blueback herring larvae reared from eggs of known parentage (Sismour and Loesch, in review). Meristic and morphometric characters suggested to be diagnostic for these species (Chambers et al. 1976) are reexamined, and pigmentation characters of potential taxonomic value are analyzed.

METHODS

Alewife and blueback herring larvae hatching from eggs of known taxonomic identity were reared to ages of 32 days and 37 days, respectively (Sismour and Loesch, in review). Larvae were subsampled throughout development, and specimens were measured with the aid of a dissection microscope fitted with a calibrated ocular micrometer. Measurements were made prior to fixation in 5% phosphate buffered formalin (5% PBF) (Markle 1984) or 95% ethanol (95% EtOH).

Formalin-fixed specimens were transferred through an increasing series of 20%, 45%, and 70% EtOH for preservation (Lavenberg et al. 1983).

Definitions of the snout to vent length and the standard length followed Lippson and Moran (1974). When referring to preflexion larvae, the term 'supracaudal' refers to the dorsum posterior to the anus in the region of the future caudal peduncle. Myomeres anterior to the cleithrum and in the posterior region of the tail were difficult to observe consistently; therefore, myomeres between the cleithrum and the anal fin insertion were enumerated. Prior to formation of the anal fin, the origin was estimated as the first postanal myomere. The number of myomeres between the dorsal fin insertion and the anal fin origin were evaluated for its potential taxonomic value. The snout-vent length to standard length (SVL/SL) ratio was calculated using live-length measurements of specimens to exclude error caused by fixative-induced shrinkage.

Melanophore distribution and morphology were examined to identify pigment characters of potential value for taxonomic delineation. Xanthophore distribution and morphology was noted when observed in specimens prior to fixation. Observations of xanthochrome were limited to anesthetized larvae since the pigment was unstable after fixation and a method to permanently record its occurrence was not available.

Multidimensional contingency table analysis (Agresti 1990) was used to test the hypothesis that myomere count was independent of species identity and standard length. Standard length was included in the analysis to determine whether an association between species identity and myomere count was consistent with increasing size of the larvae. The distributions of SVL/SL ratios for each species were analyzed using the chi-square test for independence to test the hypothesis that the distributions differed significantly.

Contingency table analysis was used to determine the significance of observed differences in the frequency of occurrence of supracaudal

melanophores between young postlarvae of the two species. The odds ratio and the associated 95% confidence interval were calculated to evaluate the likelihood that an alewife larva would demonstrate supracaudal pigmentation (Agresti 1990).

RESULTS

Eggs and Prolarvae.

Ripe, unfertilized ova and fertilized eggs of alewife and blueback herring were golden yellow. Fertilized eggs of both species readily adhered to one another and initially to nylon sieves used to rinse eggs. Blueback herring eggs were highly adhesive, remaining adhered to the sieve throughout embryonic development. Although alewife eggs remained adhered together, they became detached from sieves after several hours.

At hatching, prolarvae of both species were transparent. Embryonic pigmentation, present from hatching to about 24 hr after hatching, included pigment along the lateral surface of myomeres, below the pectoral fin buds, and on the nape. Melanophores were distributed along the ventral surface of the yolk sac and along the gut. Pectoral fin buds and the saccular and lagenar otoliths were present in both species. Eyes of both species were unpigmented at hatching, except for a scatter of golden to bronze pigment along the dorsal margin, and they were completely pigmented by the second day after hatching.

The yolk of both species was segmented, a characteristic of the Clupeidae, and oil droplets were not observed within the yolk. However, unidentified structures located between the periblast and the epiblast (Fig. 1) gave an appearance of oil globules. The chemical composition of these structures was not determined.

Postlarval Meristics and Morphometry.

A total of 64 alewife larvae from 6 mm to 18 mm SL and 70 blueback herring larvae from 6 mm to 14 mm SL were examined to determine whether the postdorsal-preanal myomere count may be used as a diagnostic taxonomic criterion for delineating larvae of these species (Table 1). Blueback herring postlarvae larger than 14 mm SL were not captured and therefore could not be included in this analysis. The hypothesis that postdorsal-preanal myomere count was jointly independent of species and standard length could not be rejected (X^2=39.7, p=0.351). This result supports the conclusion that this taxonomic characteristic does not differ significantly between alewife and blueback herring larvae, and that this characteristic is of little value as a diagnostic taxonomic criterion.

The SVL/SL ratio ranged for alewife larvae (n=226) ranged from 0.78 to 0.89 compared to 0.76 to 0.87 for blueback herring larvae (n=253). Frequency distributions of the SVL/SL ratio for alewife and blueback herring larvae are shown in Fig. 2. The percentage of alewife and blueback herring larvae with an SVL/SL ratio greater than 0.85 was 1.7% and 2.0%, respectively. The distribution of SVL/SL ratio values for blueback herring larvae differed significantly from the distribution of values observed for alewife larvae (X^2=18.37, p<0.01); however, the extensive overlap of the two distributions (Fig. 2) results in low resolution between the two distributions and an inability to discriminate specimens of these species utilizing this characteristic. The SVL/SL ratio appears to be of little value as a diagnostic taxonomic criterion for delineating alewife and blueback herring larvae.

Postlarval Pigmentation.

Melanophores in the supracaudal region were more frequent among alewife preflexion postlarvae than among blueback herring preflexion larvae (89% compared to 8%, respectively). The odds ratio calculated

for the observed frequencies was 89.9 (95% C.I.=40.4 to 164.0) which indicates that an alewife larva was 89.9 times more likely to have supracaudal pigment than a blueback herring larva. These melanophores were observable, when present, until flexion after which pigmentation in this region increased and identification of these melanophores became questionable.

Although more prevalent among alewife larvae, supracaudal pigmentation can not be used exclusively to delimit these species in field collections since it is not diagnostic and its frequency among field-collected larvae is likely to vary. The frequency of supracaudal pigmentation among a group of unidentified river herring larvae reared from field-collected eggs in 1990 was 29% (Sismour, unpublished data). The location and the time of year (Herring Creek and early May, respectively) suggest that these were most likely blueback herring larvae, and the relatively low incidence of supracaudal pigmentation supports this conclusion.

At approximately 11 mm, blueback herring larvae developed a stellate patch of pigmentation on the dorsal surface of the notochord at the level of the nape which appeared to consist of a single large melanophore, but could also be comprised of two melanophores (Fig. 3). At about 15 mm, alewife larvae developed a parallel set of melanophores (increasing from 1 to 3 pair with age) along the lateral surface of the notochord at the level of the nape (Fig. 4).

A method to permanently record the distribution and morphology of xanthophores in live specimens or to stabilize xanthochrome in fixed and preserved specimens was not available. Consequently, descriptions of xanthophore distribution are based on observations of anaesthetized larvae prior to fixation. Xanthochrome was initially observed in both species at about 11 mm to 12 mm SL. Among alewife larvae, xanthophores were initially distributed on the dorsal surface of the head and, by about 15 mm SL, were distributed on the body surface dorsal to the

lateral mid-line. Among blueback herring larvae, xanthophores were initially distributed on the head and the caudal fin at the base. Xanthochrome was observed above the vertebral column in blueback herring larvae beginning about 13 mm SL, and at about 14 mm to 15 mm SL, xanthochrome was observed at the base of the dorsal fin and along the dorsal mid-line.

Pigmentation Ontogeny.

Among both alewife and blueback herring larvae, pigmentation is present along the ventral midline anterior to the cleithrum with a variable appearance from a solid line to a disrupted line consisting of two or three pigment patches. A parallel series of melanophores with common origin at the cleithrum proceeds posteriorly along the ventral surface for a short distance where the rows diverge medially to occupy a position between the gut and myomeres, and ends at about the 14th to 17th myomere posterior to the cleithrum. Scattered, stellate melanophores subsequently occur along the junction of the gut and myomeres to the vent. A parallel row of melanophores begins along the ventral midline at about the 12th to 15th myomere posterior to the cleithrum and extends to the vent. Scattered melanophores occur posterior to the vent along the ventral surface in both species. Pigmentation typically occurred on the cleithrum anterior to the pectoral fins in both species.

During the early postlarval stage, prior to flexion, the rows of pigmentation posterior to the cleithrum varied in appearance from a complete, solid line to a broken or dashed line. The pigmentation pattern appeared to differ between alewife and blueback herring larvae as the two rows diverged toward their lateral position between the gut and myomeres. The differing patterns could be observed among larvae in which the pigmentation of the two rows was complete as well as in some larvae with disrupted pigmentation depending on its distribution. Among

blueback herring larvae, the rows of pigment diverged in a shallow arc and their junction at the cleithrum appeared curved (blunt V). Among alewife larvae, the junction of the two rows of pigment at the cleithrum had a more angular appearance and the rows of pigment were relatively straight as they diverged from the cleithrum (pointed V). The patterns appeared to be consistent, but were not always readily visible.

Except along the ventral aspect of the body and in the supracaudal region, little pigmentation was observed on preflexion larvae of either species. With the onset of flexion, additional pigmentation developed on the dorsal half of the body among both species and pigmentation on the dorsal and lateral surfaces of the gut began to increase. Fig. 5 a to d illustrate postflexion alewife larvae from 11.4 mm to 17.8 mm SL, and Fig. 6 a to d illustrate postflexion blueback herring larvae from 10.1 mm to 17.1 mm SL. These illustrations emphasize ontogenetic change in pigmentation only. Morphological and osteological characteristics, such as position and number of myomeres and the number and position of fin rays were illustrated as observed using specimens that were not cleared and stained.

Pigmentation on the head began with development of melanophores associated with the otic bulla. With ontogeny, melanophores appeared on the dorsal surface of the head and on the epaxial surface of the body. Melanophores on the head of alewife larvae were contracted and those on the head of blueback herring typically were stellate. Small, stellate melanophores developed on the mandible and maxillary of both species, and there were no noticeable differences.

After flexion, pigmentation of blueback herring larvae became heavier compared to alewife larvae. Qualitative difference in pigmentation between the two species was most prominent along the dorsal mid-line posterior to the dorsal fin; blueback herring demonstrated heavier pigmentation in this zone with relatively larger, stellate melanophores compared to melanophores observed on alewife larvae (Fig.

7). Pigmentation on the caudal fin also appeared to be slightly heavier among blueback herring, but this difference was not sufficient to delimit larvae.

DISCUSSION

Taxonomic delineation of alewife and blueback herring larvae remains problematic. Cianci (1969) reared larvae of both species from eggs and reported no significant meristic or morphometric variation. Chambers et al. (1976) compared field-collected herring larvae captured in locations where either gravid alewife or gravid blueback herring adults were captured in gillnets. The results suggested that larvae classified as alewife and blueback herring could be delimited on the basis of the postdorsal-preanal myomere number and the ratio of the snout to vent length to standard length (Chambers et al. 1976). Larvae classified as blueback herring were reported to have significantly more postdorsal-preanal myomeres (11 to 13 myomeres) compared to larvae classified as alewife (7 to 9 myomeres), and the SVL/SL ratio of larvae in the alewife group less than 14 mm SL was 0.87 compared to 0.82 for larvae in the blueback herring group of the same size (Chambers et al. 1976). Differences were also reported in the number of preanal myomeres and in the ratios of vent to urostyle length to standard length and vent to tail length to standard length (Chambers et al. 1976). All the morphometric measurements identified by Chambers et al. (1976) are associated with gut length, such that a longer gut yields a greater preanal myomere count and postdorsal-preanal myomeres and in a higher SVL/SL ratio.

Other studies that have compared either alewife or blueback herring larvae to gizzard shad larvae do not support the conclusions suggested by Chambers et al. (1976). Lam and Roff (1977) compared alewife larvae and gizzard shad larvae from Lake Ontario and reported results similar to Bulak (1985) who compared postlarval blueback herring

to postlarval gizzard shad and postlarval threadfin shad in the Santee-Cooper drainage of South Carolina. According to Bulak (1985), blueback herring greater than 16 mm SL are delimited by an anal fin ray (pterygiophore) count less than 18 compared to 23 or more for the gizzard and threadfin shads of the same length. Blueback herring larvae less than 14 mm SL were typically characterized by fewer than 10 postdorsal-preanal myomeres (range: 5-11) compared to greater than 10 postdorsal-preanal myomeres (range 10-14) for gizzard shad larvae of the same length (Bulak 1985). The only reliable characteristic that Lam and Roff (1976) identified that delimited alewife and gizzard shad larvae less than 16 mm SL was the SVL/SL ratio. For larvae less than 16 mm SL, the SVL/SL ratio for alewife ranged from 0.78 to 0.85 and for gizzard shad ranged from 0.85 to 0.88. Alewife and gizzard shad larvae of 16+ mm SL exhibited SVL/SL ratios from 0.78 to 0.82 and from 0.75 to 0.86, respectively (Lam and Roff 1977).

Bulak (1985) suggested that Chambers et al. (1976) may have reversed the identification of the alewife and blueback herring groups. However, the findings of both Lam and Roff (1977) and Bulak (1985) suggest that Chambers et al. (1976) may have compared river herring larvae to gizzard shad larvae.

Bulak (1985) and Lam and Roff (1977) support Cianci's (1969) conclusion that morphometric and meristic characters of alewife and blueback herring larvae do not differ significantly. The present study also supports this conclusion, but evidence suggests that pigmentation characters differ between the two taxa. Pigmentation characters which delimit postflexion larvae developed as early as about 12 mm, prior to formation of juvenile characteristics. Pigmentation on the notochord at approximately at the level of the nape appears to be diagnostic. Other pigmentation characters, which depend on qualitative differences in the amount of pigmentation or on melanophore morphology, may assist in

species identification prior to the complete development of adult characters.

Xanthophore distribution appears to provide another potential character to delimit field-collected river herring larvae. However, its utility as a taxonomic character is currently limited due to an inability to stabilize this pigment in fixed and preserved specimens. Additional study is necessary to validate the occurrence of this pigmentation in field-collected specimens, and whether its occurrence varies between river systems or locations within the nursery zone. Xanthochrome may be more prevalent among populations of clupeid larvae that inhabit highly turbid systems (e.g. tidal freshwater) compared to systems of lower turbidity. In highly turbid water, the yellow component of the visible light spectrum is the least attenuated fraction (Pickard and Emery 1982). In systems characterized by high turbidity, xanthochrome be important as disruptive pigmentation facilitating predator-avoidance by clupeid larvae.

Although the argument has been made that pigmentation patterns are of little value for identifying clupeid species (e.g. Russell 1976), pigment characters may aid the identification of larvae of some groups prior to development of complete osteological characteristics or when meristic and morphometric characters overlap. Recent studies have provided a basis for using pigment characters in establishing taxonomic relationships among early life stages of fishes (Kendall et al. 1983). Further studies of pigment characters of clupeid larvae may be of value to facilitate the identification of field-collected specimens.

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LITERATURE CITED

Agresti, A. 1990. Categorical data analysis. J. Wiley and Sons, New York, New York. 558 p.

Ahlstrom, E. H. and H. G. Moser. 1976. Eggs and larvae of fishes and their role in systematic investigations and in fisheries. Revue des Travaux de l'Institute des Peches maritimes 40: 379-398.

Berry, F. H. and W. J. Richards. 1973. Characters useful to the study of larval fishes, pages 48-65. *In*: Proceedings of a workshop on egg, larval and juvenile fish in Atlantic coast estuaries. National Marine Fisheries Service, Mid-Atlantic Fisheries Center, Technical Publication Number 1. 338 p.

Boreman, J. 1981. Life histories of seven fish species that inhabit the Hudson River estuary. National Marine Fisheries Service, Northeast Fisheries Center, Woods Hole Laboratory, Laboratory Reference Document Number 81-34. 97 p.

Bulak, J. S. 1985. Distinction of larval blueback herring, gizzard shad, and threadfin shad from the Santee-Cooper drainage, South Carolina. Journal of the Elisha Mitchell Scientific Society 101: 177-186.

Cianci, J. M. 1969. Larval development of the alewife *Alosa pseudoharengus* Wilson, and the glut herring, *Alosa aestivalis* Mitchill. M.S. Thesis, University of Connecticut, Storrs, Connecticut. 62 p.

Chambers, J. R., J. A. Musick, and J. Davis. 1976. Methods of distinguishing larval alewife from blueback herring. Chesapeake Science 17: 93-100.

Dovel, W. L. 1971. Fish eggs and larvae of the upper Chesapeake Bay. Contribution Number 460, National Resources Institute, University of Maryland. 71 p.

Dovel, W. L., A. J. Mansueti, and E. H. Oldmixon. 1965. Notes on the identification of five species of juvenile herring, with a new artificial key to the species. Chesapeake Biological Laboratory, Solomons, Maryland, Reference 65-101. 13 p.

Funes-Rodriguez, R. and A. Esquivel-Herrera. 1985. Determinacion de las principales caracteristicas que permiten la identificacion de las larvas de *Opisthonema* spp. en la costa del pacifico de Baja California Sur. Investigacions Marinae, CICIMAR 2: 78-85.

Houde, E. D. and P. L. Fore. 1973. Guide to identity of eggs and larvae of some Gulf of Mexico clupeid fishes. Florida Department of Natural Resources, Marine Research Laboratory, Leaflet Series 4 (part 1, no. 23). 14 p.

Kendall, A. W., Jr., E. H. Ahlstrom, and H. G. Moser. 1983. Early life history stages of fishes and their characters, pages 11-22. *In*: H. G. Moser et al. (editors), Ontogeny and systematics of fishes. Special Publication Number 1, American Society of Ichthyologists and Herpetologists, Allen Press, Inc., Lawrence, Kansas. 760 p.

Kuntz, A., and L. Radcliffe. 1917. Notes on the embryology and larval development of twelve teleostean fishes. United States Bureau for Fisheries, Bulletin 35: 87-134.

Lam, C. N. H., and J. C. Roff. 1977. A method for separating alewife *Alosa pseudoharengus* from gizzard shad *Dorosoma cepedianum* larvae. Journal of Great Lakes Research 3: 313-316.

Lavenberg, R. J., G. E. McGowen and R. E. Woodsum. 1983. Preservation and curation, pages 57-59. *In*: H. G. Moser et al. (editors), Ontogeny and systematics of fishes. Special Publication Number 1, American Society of Ichthyologists and Herpetologists, Allen Press, Inc., Lawrence, Kansas. 760 p.

Lippson, A. J. and R. L. Moran (eds.). 1974. Manual for identification of early developmental stages of fishes of the Potomac River estuary. Maryland Power Plant Siting Prog., Miscellaneous Publication Number 13. 282 p.

Loesch, J. G. 1987. Overview of life history aspects of anadromous alewife and blueback herring in freshwater habitats. American Fisheries Society Symposium 1: 89-103.

McGowan, M. F. and F. H. Berry. 1983. Clupeiformes: development and relationships, pages 108-126. *In*: H. G. Moser et al. (editors), Ontogeny and systematics of fishes. Special Publication Number 1, American Society of Ichthyologists and Herpetologists, Allen Press, Inc., Lawrence, Kansas. 760 p.

Mansueti, R. J. 1956. Alewife herring eggs and larvae reared successfully in lab. Maryland Tidewater News 13: 2-3.

Markle, D. F. 1984. Phosphate buffered formalin for long term preservation of formalin fixed ichthyoplankton. Copeia 1984: 525-528.

Norden, C. R. 1967. Development and identification of the larval alewife, Alosa pseudoharengus (Wilson), in Lake Michigan. Journal of Great Lakes Research 10: 70-78.

Pickard, G. L. and W. J. Emery. 1982. Descriptive physical oceanography: An introduction. 4th (SI) enlarged edition. Pergamon Press, Oxford. 249 p.

Powles, H. and D. F. Markle. 1983. Identification of larvae, pages 31-33. *In*: H. G. Moser et al. (editors), Ontogeny and systematics of fishes. Special Publication Number 1, American Society of Ichthyologists and Herpetologists, Allen Press, Inc., Lawrence, Kansas. 760 p.

Richards, W. J., R. V. Miller, and E. D. Houde. 1974. Egg and larval development of the Atlantic thread herring, *Opisthonema oglinum*. Fishery Bulletin, U.S. 72: 1123-1136.

Russell, F. S. 1976. The eggs and planktonic stages of British marine fishes. Academic Press, London. 524 p.

Taber, C. A. 1969. The distribution and identification of larval fishes in the Buncombe Creek arm of Lake Texoma with observation on spawning habits and relative abundance. Ph.D. thesis, University of Oklahoma, Oklahoma. (Not seen).

Wang, J. C. S. 1970. Characters of, and keys to, young clupeids collected in the Delaware River in the vicinity of Artificial Island, pages 154-172. *In*: V. J. Schuler et al. (editors), Ecological study of the Delaware River in the vicinity of

Artificial Island. Progress Report., PSE&G Co., January-December 1969, Part. 2., Ichthyological Associates, Middletown, Delaware. 426 p.

Wang J. C. S. and R. J. Kernehan. 1979. Fishes of the Delaware estuaries: a guide to early life histories. Ecological Analysts, Towson, Maryland. 410 p.

Table 1. Postdorsal-preanal myomere counts of alewife and blueback herring postlarvae with standard length.

Standard Length (mm)	Species	Postdorsal-preanal Myomere Count						
		5	6	7	8	9	10	11
6	Alewife						1	
	Blueback				1	1	1	
7	Alewife					2		
	Blueback				1	7	2	
8	Alewife					1	1	
	Blueback					1	1	
9	Alewife				1	1		
	Blueback					3	1	2
10	Alewife					2	4	
	Blueback				1	4	2	1
11	Alewife					1	5	
	Blueback			1		5		1
12	Alewife			1		1	4	
	Blueback			4	5	5	2	1
13	Alewife			2	1	10		
	Blueback			6	7	3		1
14	Alewife			1				
	Blueback		1	1	4			
15	Alewife			3		2		
	Blueback							
16	Alewife		1	2	3			
	Blueback							
17	Alewife	1	3		1			
	Blueback							
18	Alewife		2		1			
	Blueback							

Figure 1. Photomicrograph of an *Alosa* prolarva emphasizing unidentified structures (U) associated with the yolk sac membranes. Structures also identified include: eye (E), heart (H), otoliths (O), and yolk (Y). The specimen was fixed and preserved in 95% EtOH causing the yolk to dehydrate and withdraw from the membrane which facilitated observation of the unidentified structures.

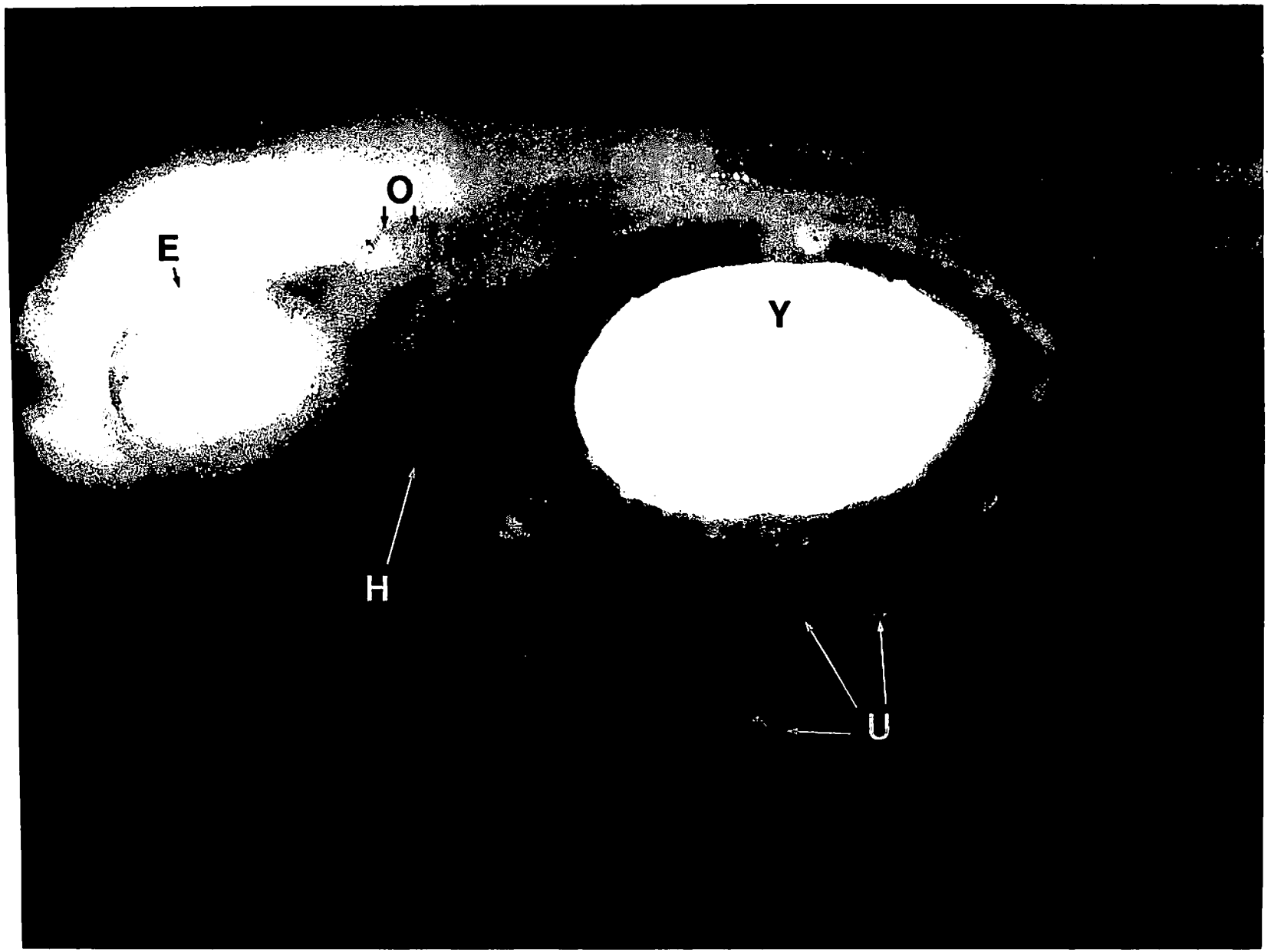

O
E
Y
H
U

Figure 2. Frequency distributions of the ratio of vent length to standard length for cultured specimens of alewife and blueback herring postlarvae.

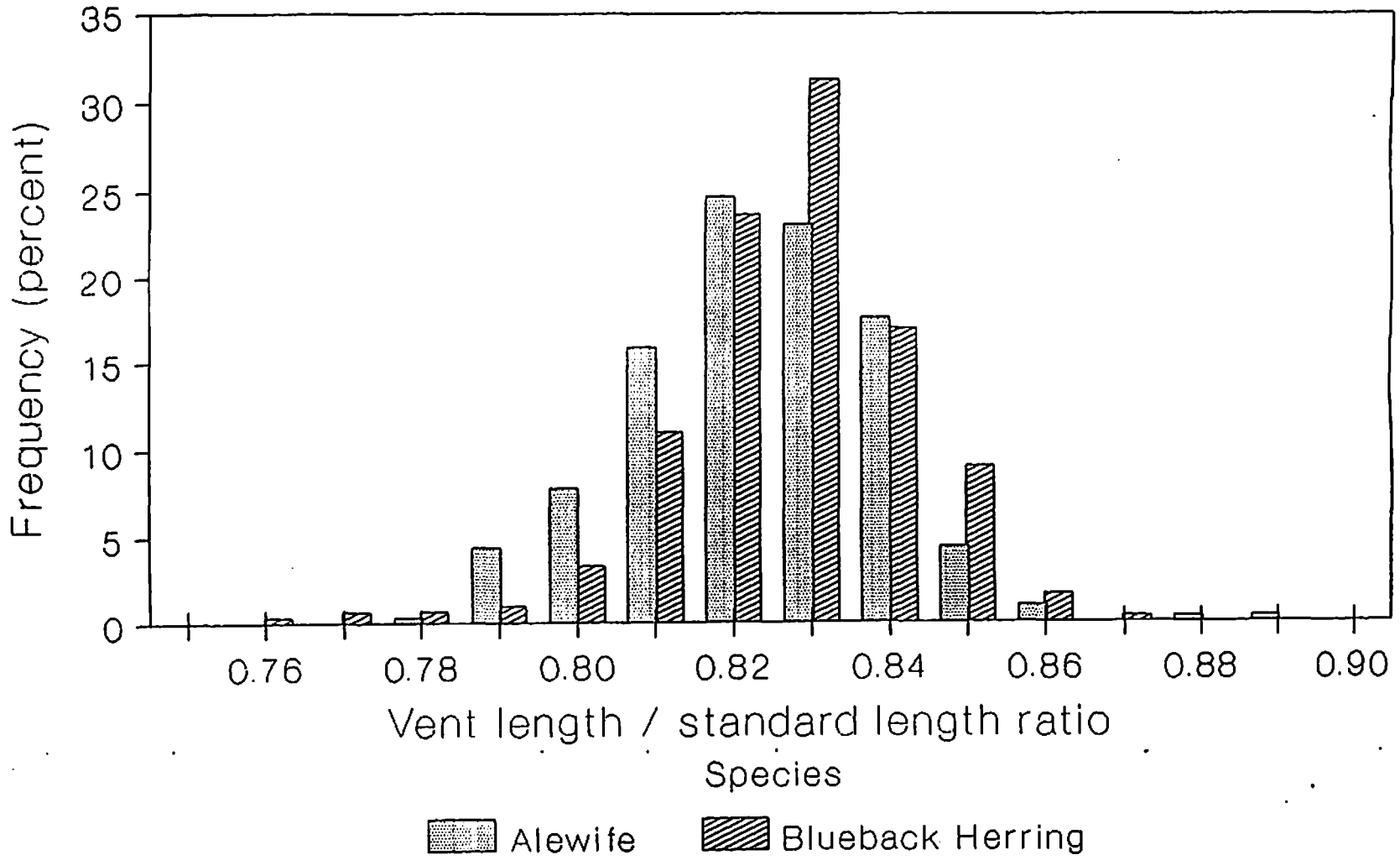

35
30
25
20
15
10
5
0
Frequency (percent)
0.76
0.78
0.80
0.82
0.84
0.86
0.88
0.90
Vent length / standard length ratio
Species
Alewife
Blueback Herring

Figure 3. Subdermal pigmentation on the dorsal aspect of the notochord of cultured blueback herring postlarvae at the level of the nape. The illustrations demonstrate two alternative patterns observed among specimens: A) single melanophore pattern, and B) double melanophore pattern. Dermal pigmentation in the head region is illustrated.

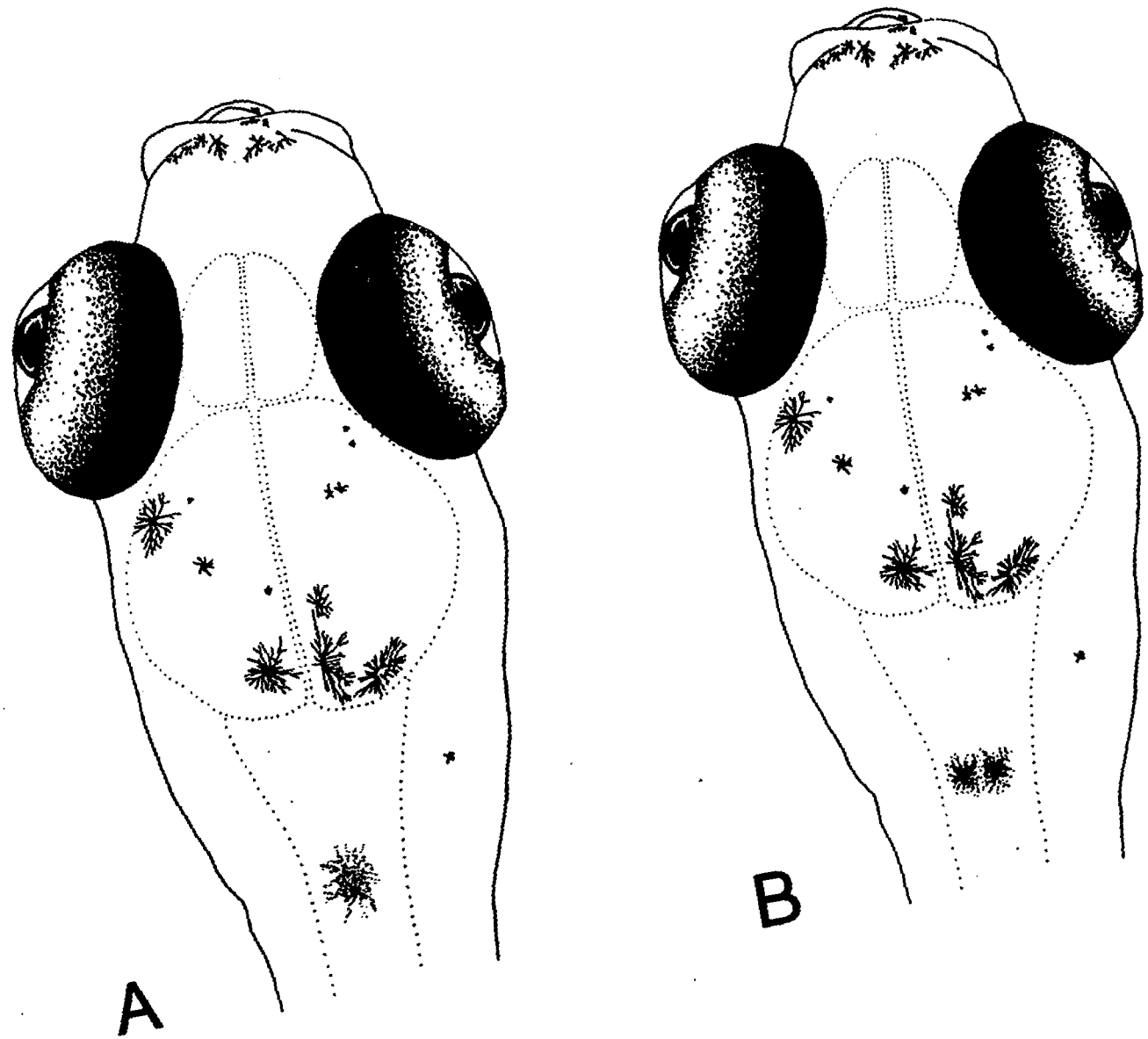

A
B

Figure 4. Subdermal pigmentation on the lateral aspect of the notochord of cultured alewife postlarvae at the level of the nape. The illustrations demonstrate the increase in melanophores along the lateral surface of the notochord that occurred with ontogeny: A)younger larva, and B) older larva. Dermal pigmentation in the head region is illustrated.

A
B

Figure 5 (a). Cultured alewife larva, 11.4 mm SL

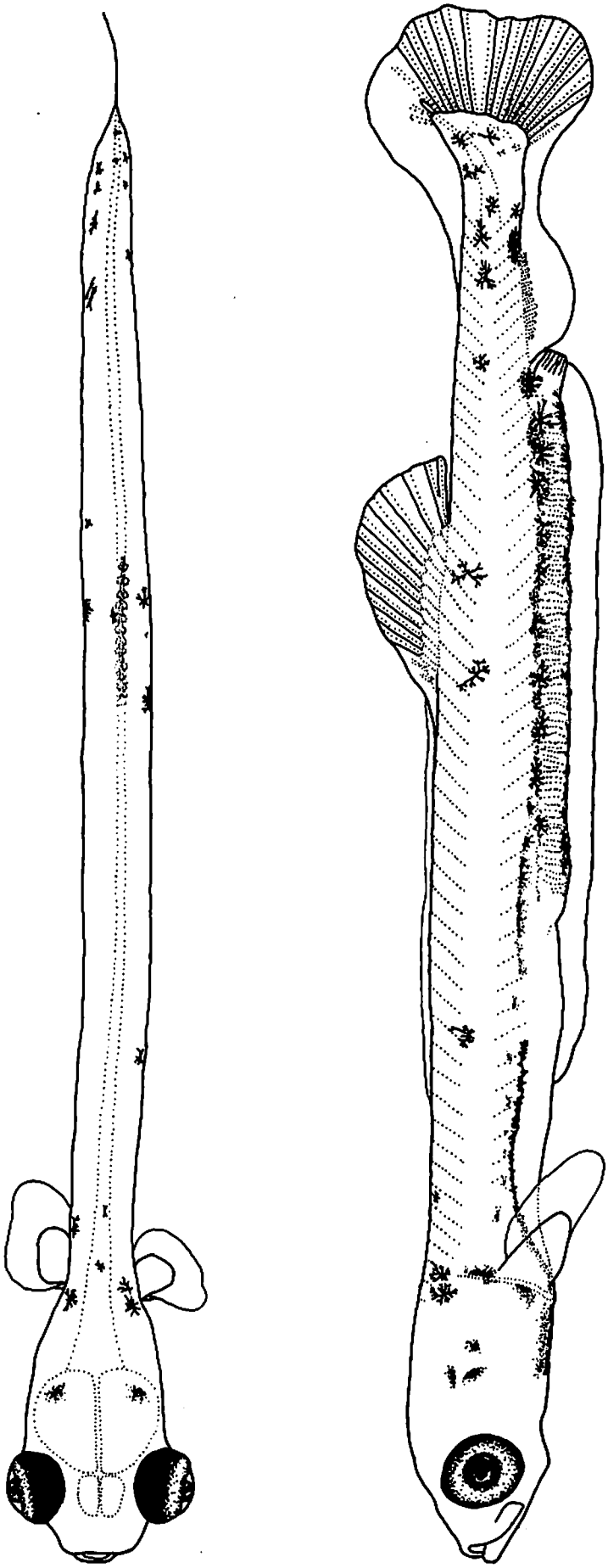


Figure 5 (b). Cultured alewife larva, 13.3 mm SL

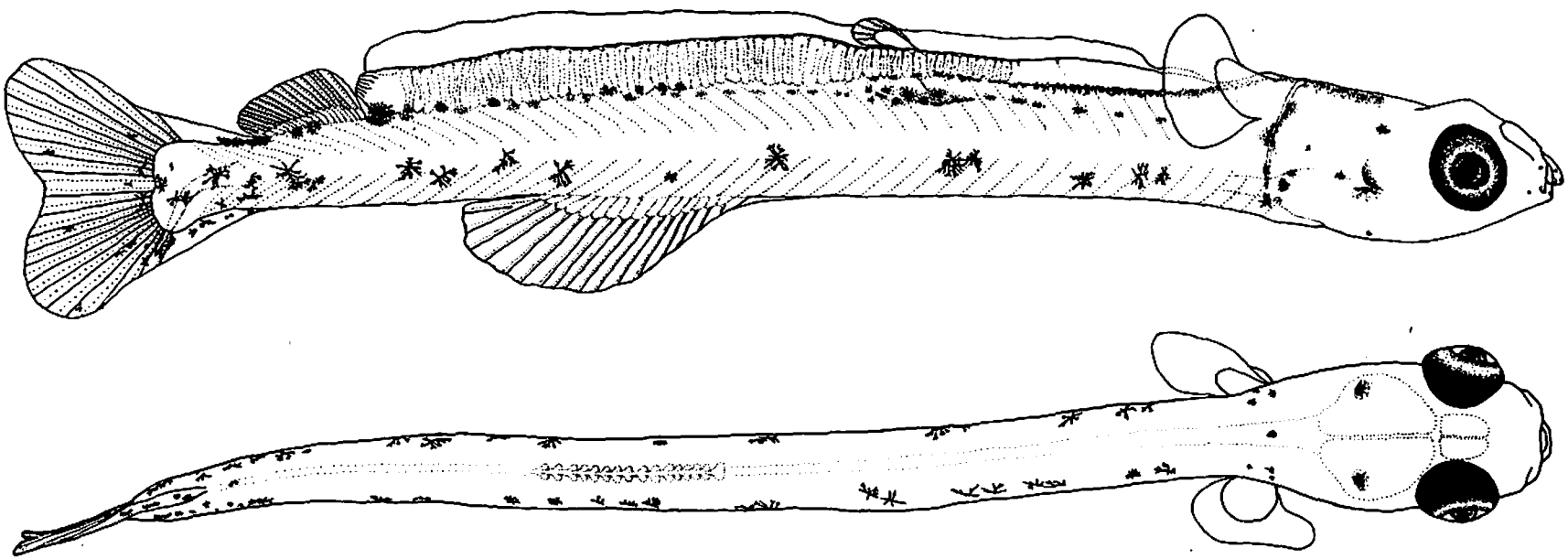


Figure 5 (c). Cultured alewife larva, 15.2 mm SL

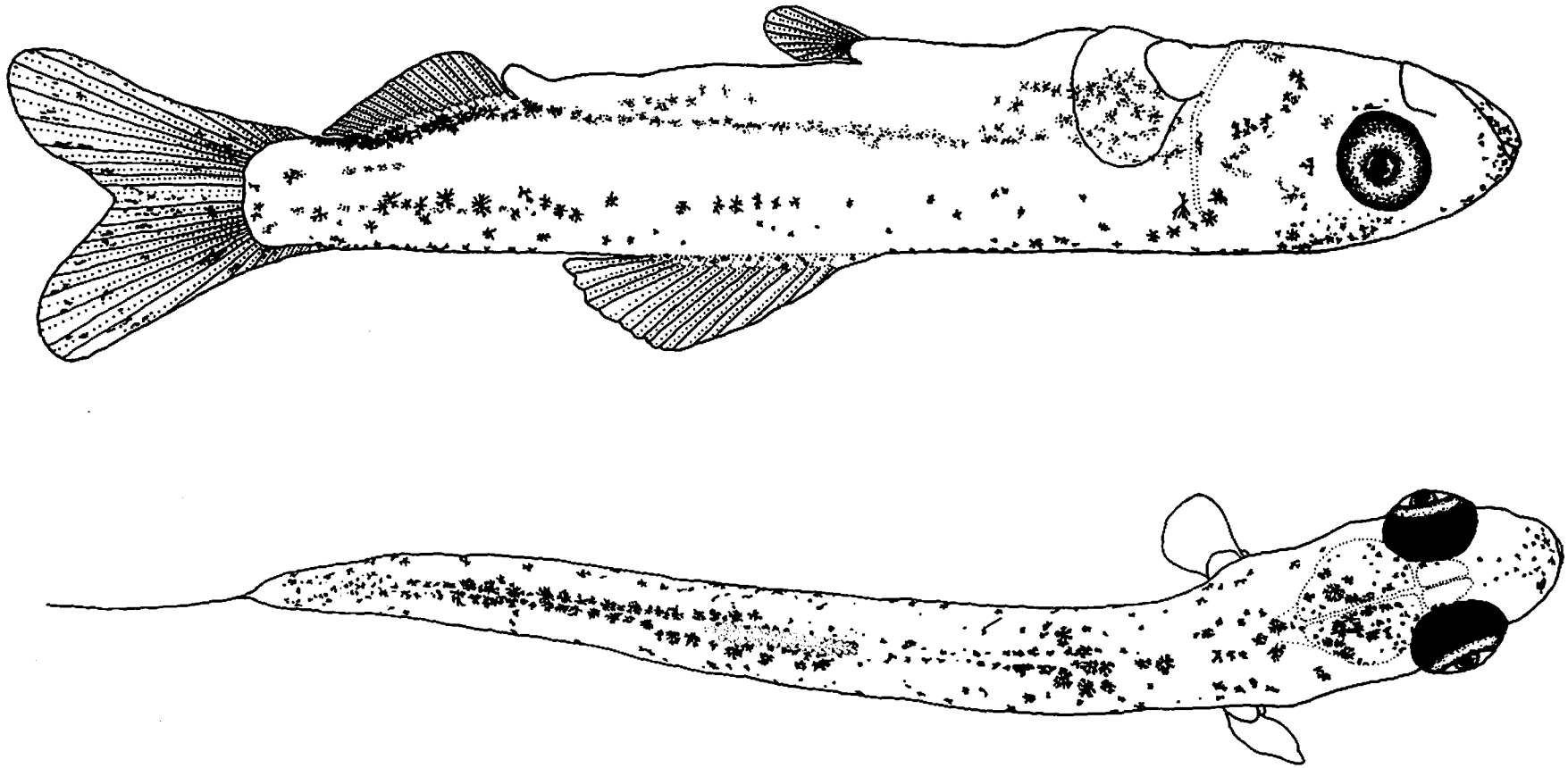


Figure 5 (d). Cultured alewife larva, 17.8 mm SL

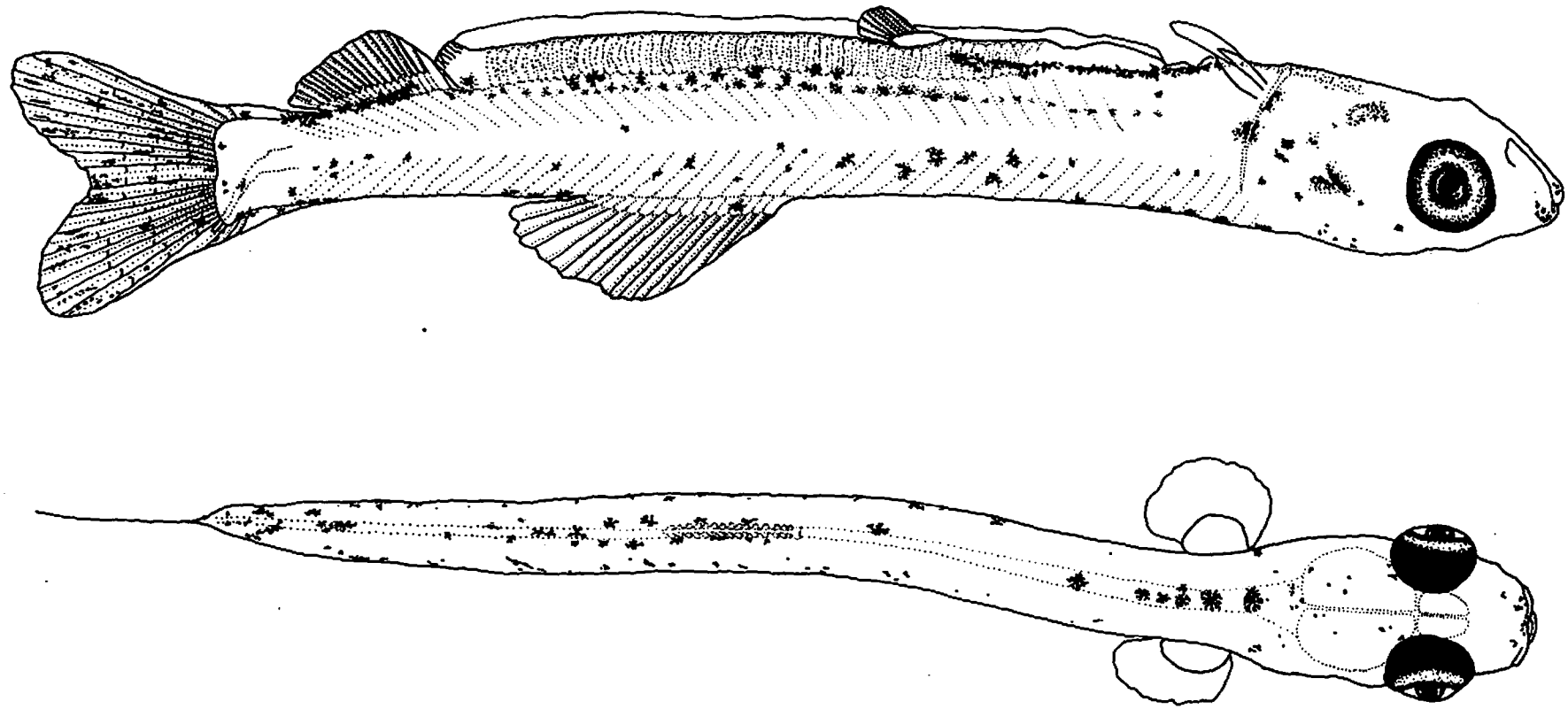


Figure 6 (a). Cultured blueback herring larva, 10.1 mm SL

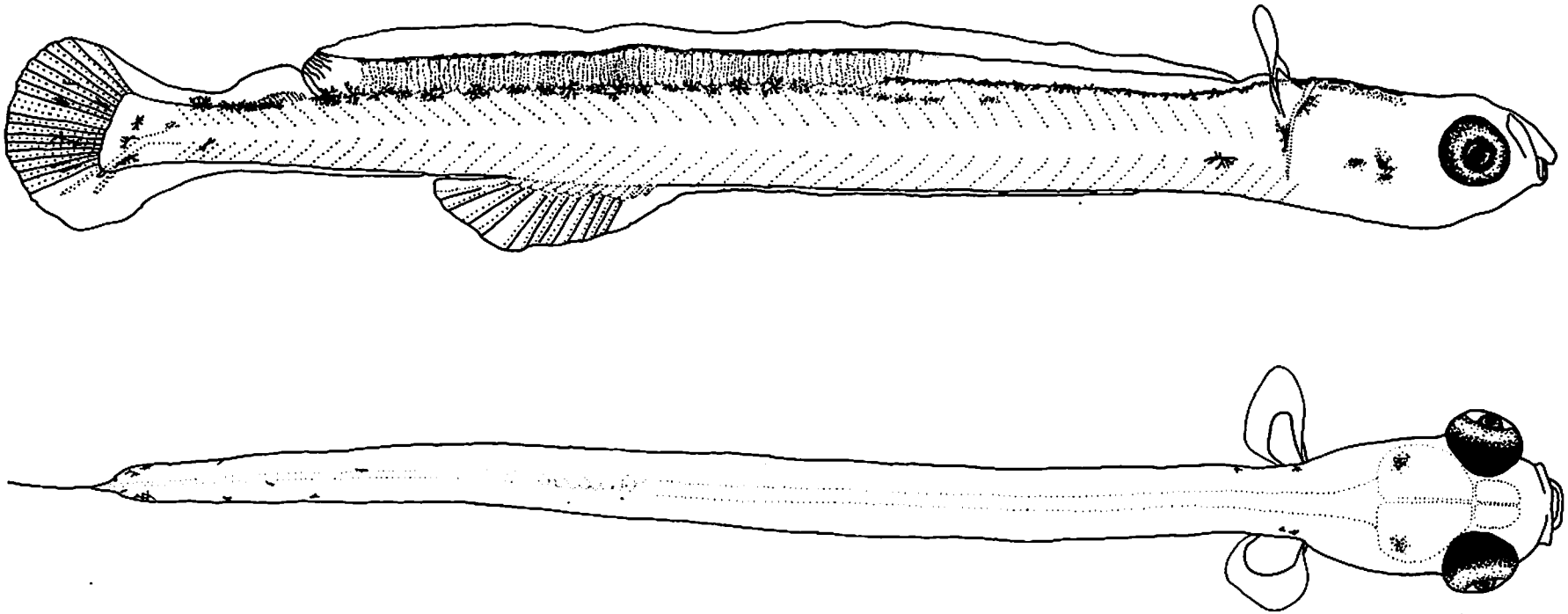


Figure 6 (b). Cultured blueback herring larva, 11.6 mm SL

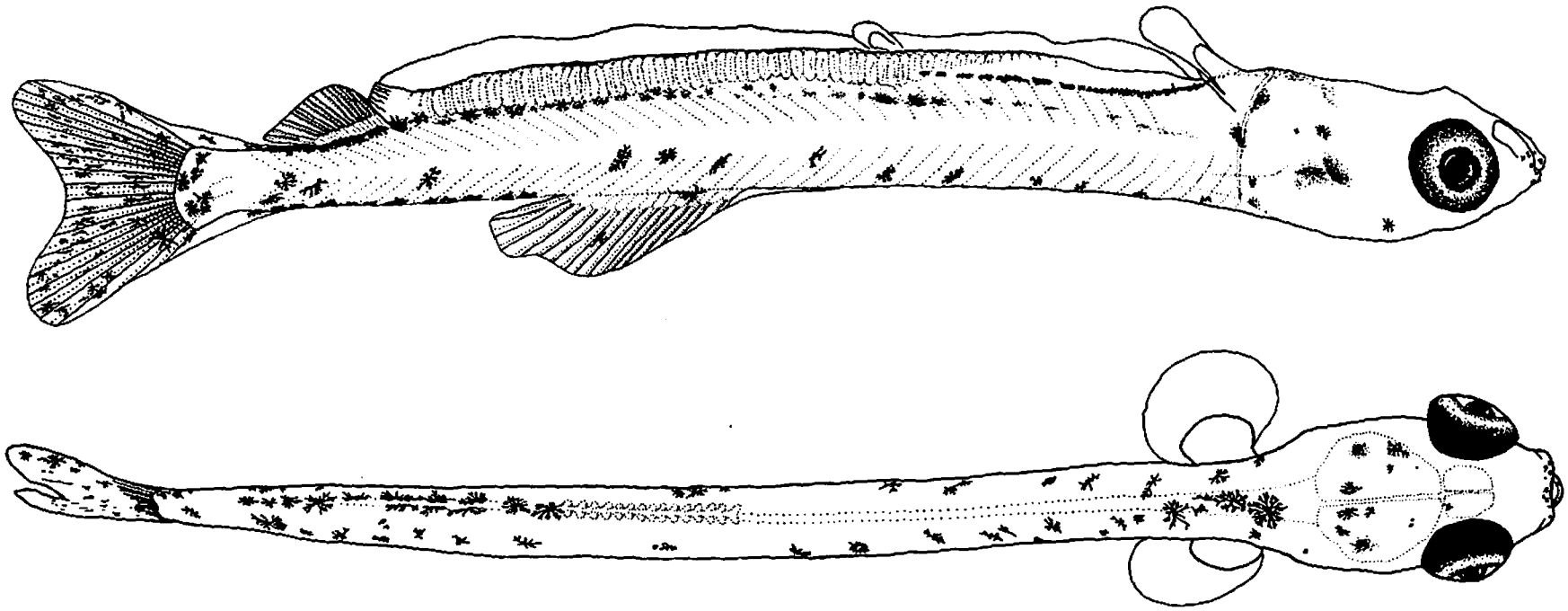


Figure 6 (c). Cultured blueback herring larva, 13.5 mm SL

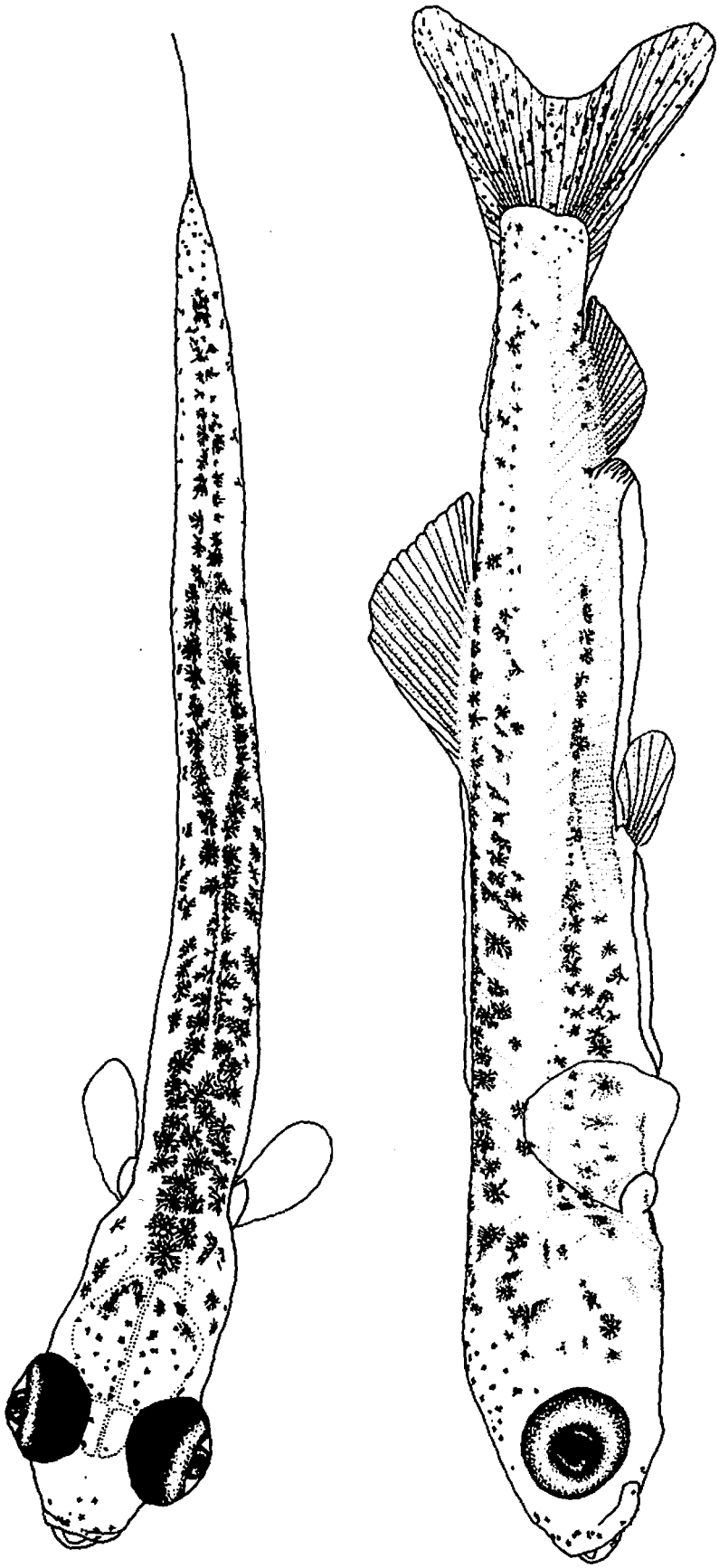
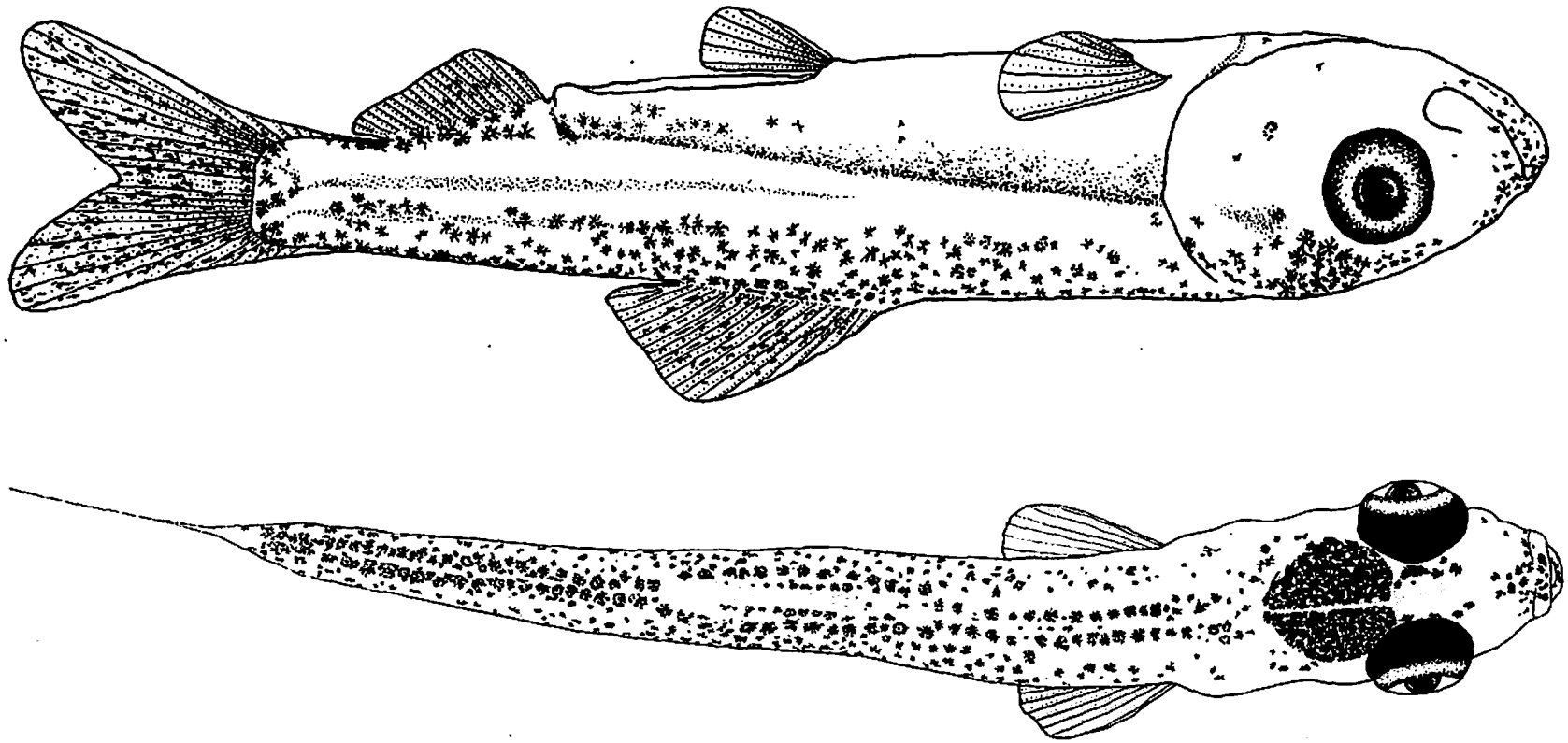


Figure 6 (d). Cultured blueback herring larva, 17.1 mm SL



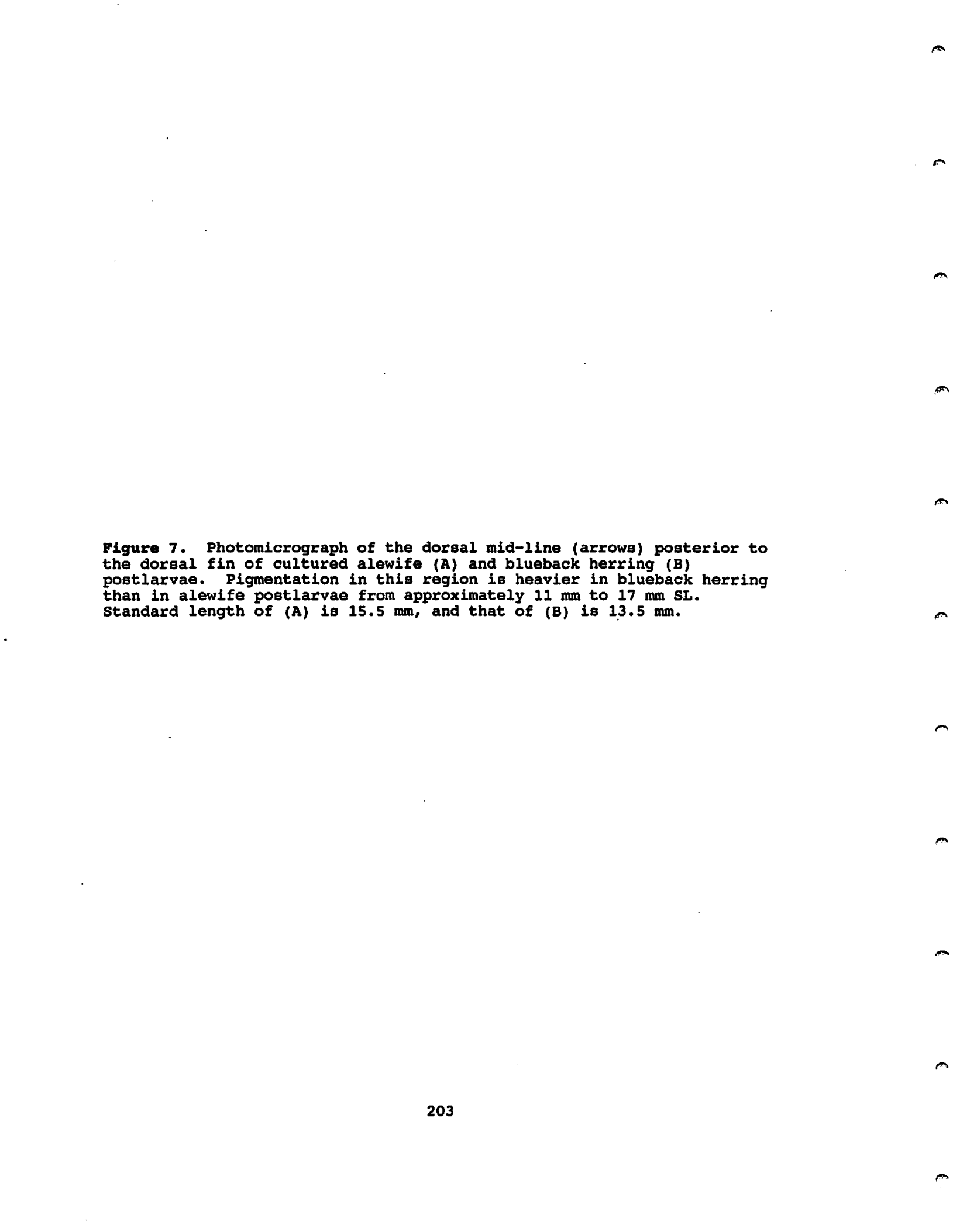
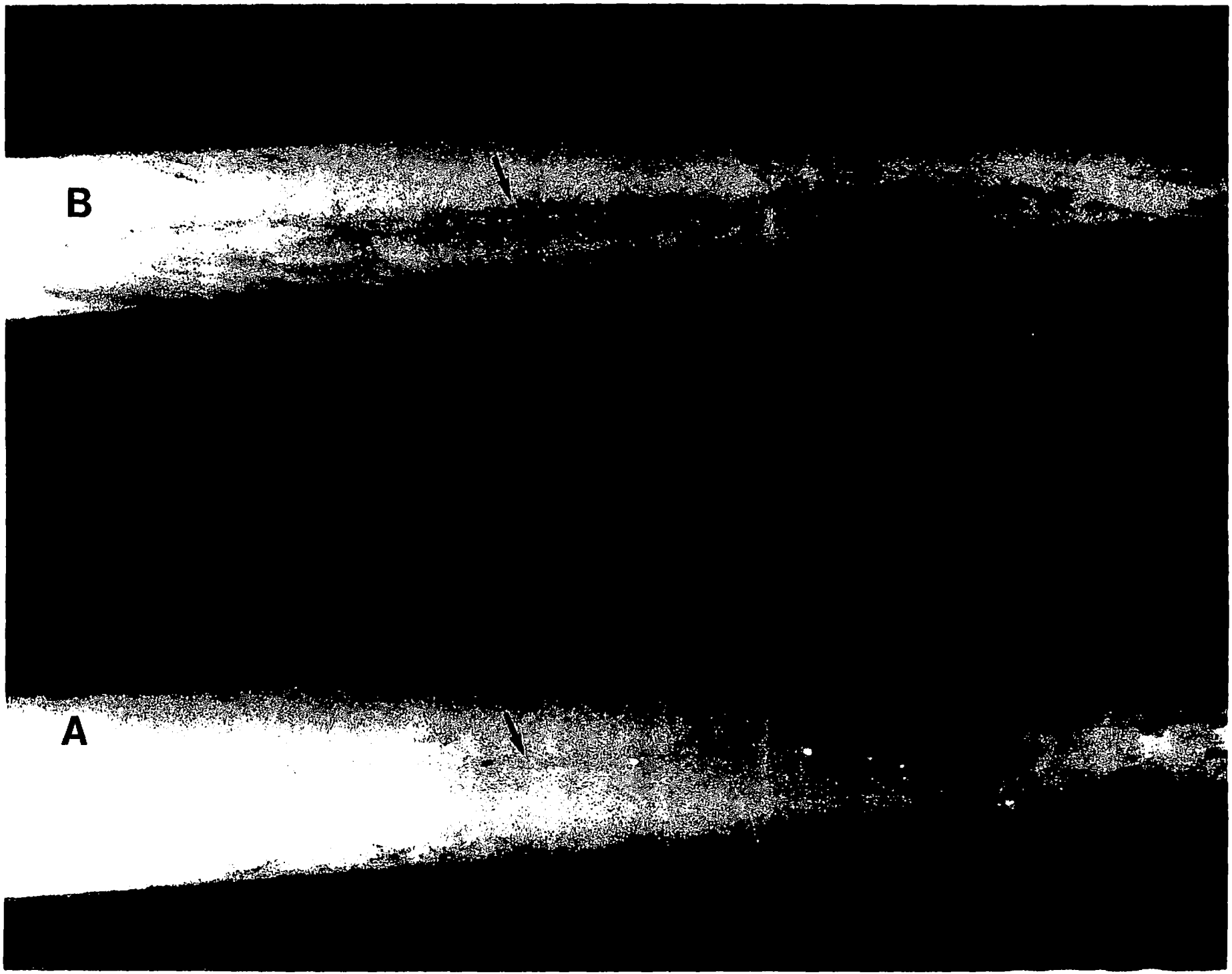


Figure 7. Photomicrograph of the dorsal mid-line (arrows) posterior to the dorsal fin of cultured alewife (A) and blueback herring (B) postlarvae. Pigmentation in this region is heavier in blueback herring than in alewife postlarvae from approximately 11 mm to 17 mm SL. Standard length of (A) is 15.5 mm, and that of (B) is 13.5 mm.


B
A