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DEVELOPMENT OF FED AND STARVED STRIPED BASS (*Morone saxatilis*) LARVAE FROM THE ROANOKE RIVER, NORTH CAROLINA

Completion Report for ECU Grant/Contract No. 5-21431

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ABSTRACT

Fed and unfed striped bass larvae were reared in a laboratory using ambient Roanoke River water to determine differences in growth and development. These differences were used to histologically determine the nutritional state of Roanoke River striped bass larvae collected from the Roanoke River delta and western Albemarle Sound from 1984 through 1986. An additional experiment examined the effects of high concentrations of aluminum in acidified waters on larval striped bass skin. Larvae that were given Artemia as a food source were successful at feeding by 6.5 days post-hatch. Decline in nutritional state of unfed larvae was apparent as soon as 5.5 days post-hatch. The organs and tissues showing signs of poor nutritional state were the eyes, liver, digestive tract, kidney, and trunk musculature. The pancreas may also be an indicator organ but results were not conclusive. Changes in these tissues occurred within 11.5 days post-hatch. Unfed larvae showed abnormal eye development by 7.5 days post-hatch. The livers of fed larvae showed moderate multifocal glycogen accumulation, while unfed larvae showed reduction in liver glycogen by 6.5 days post-hatch. Fed and unfed larvae possessed yolk 4.5 days after hatching; the rate of yolk absorption was highly variable in both groups. Fed larvae had distended, thick-walled digestive tracts; the guts of unfed larvae were collapsed and empty as soon as 4.5 days post-hatch. The hemopoietic tissue in the pronephric kidneys was reduced in unfed larvae 5.5 days post-hatch and older. Fed larvae had thickened, well-developed muscle fibers, while muscles of unfed larvae appeared thinner and separated as soon as 6 days post-hatch. Wild Roanoke River larvae examined histologically showed normal tissue development, indicating that they were not in a starving condition. It is quite likely, however, that larvae dying from or weakened by starvation are easily preyed upon or are not susceptible to net capture. Results of the pH-aluminum experiment indicated that the microridge structure of the larval epidermis was severely altered in the presence of low pH (5.5) and high aluminum (680 ug Al^{3+}/l). Our results suggest that Roanoke striped bass larvae may suffer high mortality one to two days post-hatch from skin stress, followed by a second mortality from starvation.

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INTRODUCTION

Striped bass (Morone saxatilis) inhabiting Albemarle Sound and its tributaries support important commercial and recreational fisheries in coastal North Carolina. The major spawning area for Albemarle Sound striped bass is located in the Roanoke River, which discharges into the extreme western end of Albemarle Sound approximately seven river miles (RM) downstream from Plymouth, North Carolina (Figure 1). The primary spawning ground is upstream between the towns of Halifax (RM 120) and Weldon (RM 130), North Carolina. The historical spawning grounds further upstream were blocked by construction in 1955 of the Roanoke Rapids Dam at RM 137 (McCoy 1959). Spawning occurs from late April through early June (Hassler et al. 1981); peak spawning activity is associated with water temperatures near 20oC (McCoy 1959). Eggs develop to the hatching stage as they are transported downstream by currents. After hatching, the larvae continue to be swept downstream through the Roanoke River delta to the historical nursery grounds in western Albemarle Sound (Street 1975).

In recent years, the striped bass fishery in this area has suffered from reduced numbers of harvestable adults. The population decline may be due to a variety of factors such as reduced egg viability (Guier et al. 1980, Hassler et al. 1981), poor survival of larvae during downstream drift from spawning grounds to nursery areas (Rulifson 1984, Rulifson and Stanley 1985; Rulifson et al. 1986), and poor survival of juveniles on the nursery grounds (Hassler et al. 1981). Estimates of the number of eggs spawned each year in the Roanoke River indicate that adequate numbers of eggs and larvae are produced to maintain population levels (Kornegay 1981, Kornegay and Mullis 1984). However, surveys of larvae and early juveniles in nursery areas of the western Sound indicate low recruitment of these life stages (Rulifson 1984; Rulifson, Stanley and Cooper, unpublished data). Thus, extensive mortality may be occurring in the lower Roanoke River.

Starvation of larvae has been hypothesized as one of the principal causes of mortality (Rulifson 1984; Rulifson and Stanley 1985; Rulifson et al. 1986). Striped bass larvae appear to be food limited in the Roanoke River system in years of high flow and extremely low flow (Rulifson et al.

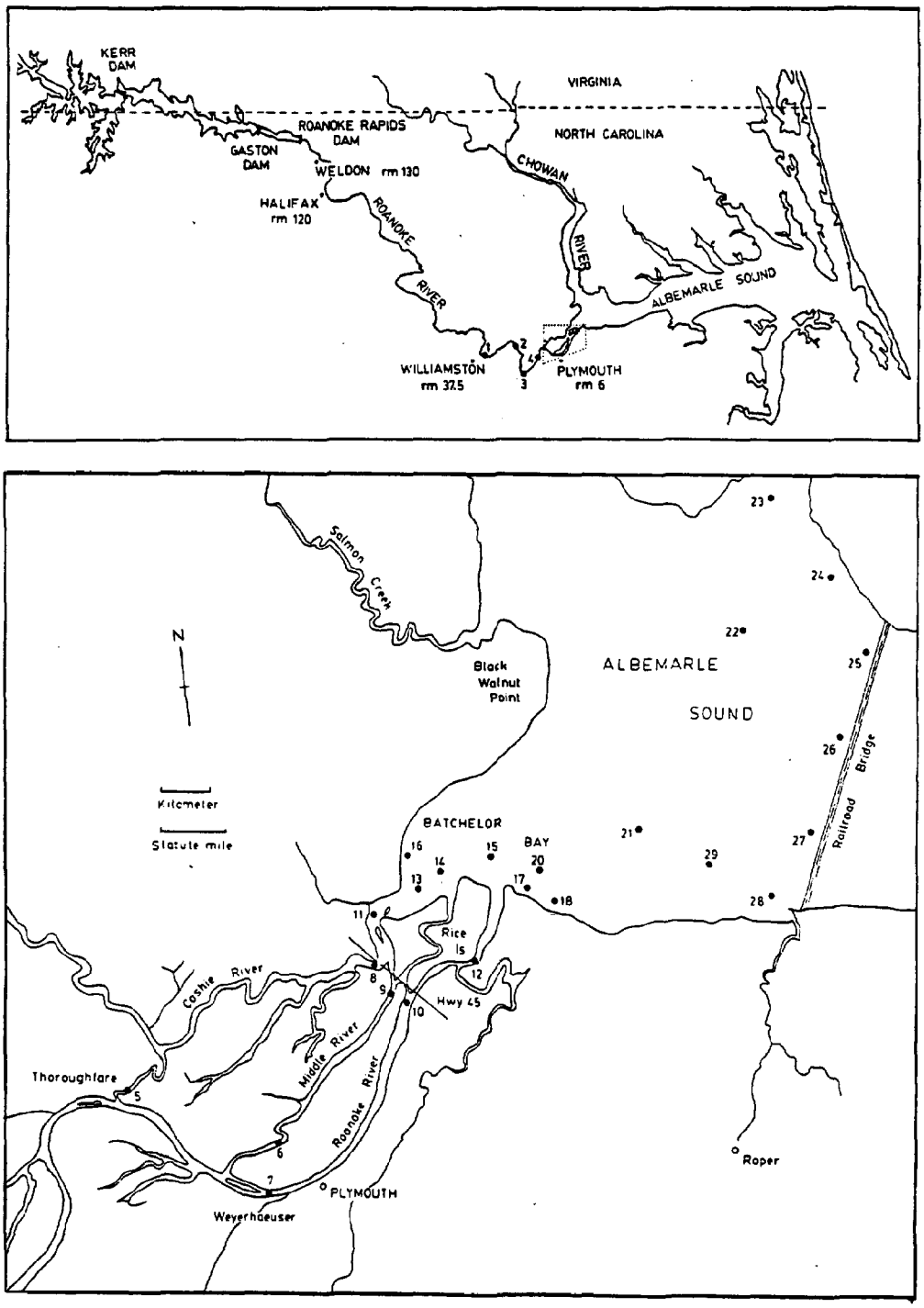


Figure 1. Map of the Roanoke River and western Albemarle Sound, North Carolina, showing Weldon (rearing experiments) and sampling locations for wild striped bass larvae in 1984, 1985, and 1986.

1986). High river flow, caused by freshwater discharge from Roanoke Rapids Lake, sweeps striped bass eggs and yolk-sac larvae into areas of extremely poor zooplankton productivity in western Albemarle Sound (Rulifson and Stanley 1985). Low flow conditions allow greater zooplankton productivity in the lower Roanoke River, but not in concentrations great enough for larvae to feed successfully (Rulifson et al. 1986). Poor water quality or the presence of pollutants, possibly causing aberrant feeding behavior of the larvae and resulting in starvation, has been hypothesized also (Rulifson 1984). Further changes in water quality and flow conditions associated with the proposed Lake Gaston pipeline to Virginia Beach and new pulp mills proposed for construction below the striped bass spawning grounds may aggravate these problems to the point at which damage to the striped bass population will be beyond its natural potential for recovery.

The goal of this study was to determine the nutritional state of Roanoke striped bass larvae. The work was designed in a manner comparable to research conducted in Maryland to examine starvation of Potomac River striped bass larvae (Martin and Malloy 1981). Specific objectives of our study were: (1) to create a histological grading scale of nutritional state in larvae; (2) to utilize this scale to estimate the nutritional state of Roanoke River larvae; and (3) to relate the findings to ongoing larval striped bass and food availability studies in the Roanoke River and western Albemarle Sound.

METHODS

Starvation Experiments

Rearing and starvation experiments of striped bass larvae were conducted from 23 April to 8 May 1985 at the State hatchery in Weldon, North Carolina. Parental stock was captured by electroshocking from the Dan River, Virginia (part of the Roanoke River watershed above the John H. Kerr Reservoir), and transported by tank truck to the Weldon hatchery. Striped bass were spawned according to procedures described by Bonn et al. (1976).

Experiment 1. Eggs of two female striped bass were fertilized with a mixture of milt taken from several males and placed in MacDonald hatching jars. The water source was unfiltered ambient water pumped from the Roanoke River into the hatchery using a once-through system. Hatching larvae were allowed to swim into aquaria. After two days they were transferred to experimental tanks at an estimated density of 1500 larvae per tank (87 larvae/l). Each rearing tank was constructed of a five-gallon plastic pickle bucket with a 14-mm I.D. PVC standpipe to control water level. The standpipe was covered by a 52-mm I.I PVC pipe fitted with a series of holes and screened with 250-um nitex mesh to allow the maximum amount of water to drain from the tank throughout the water column with a minimal velocity at any given point. Water flow into each tank was maintained at approximately 53 ml/second ($\pm$10.6, n=36) for the duration of the experiment. Complete water exchange occurred about every 5.4 minutes. Freshly hatched brine shrimp (*Artemia*) nauplii were fed to the larvae at uniform intervals throughout the day, and approximately one hour prior to procuring daily subsamples of the larvae for examination. Larvae were sampled every 24 hours, placed on ice for 10 minutes to minimize regurgitation and body flexure, and preserved in 10% buffered formalin. Only live larvae were preserved so that starvation effects would not be confused with postmortem lysing. Each larva was measured to the nearest 0.1 mm total length (TL) using an ocular micrometer.

Experiment 2. The eggs of one female striped bass were fertilized with a mixture of milt from several males and allowed to hatch in the manner described above. Two-day old larvae were transferred to the rearing tanks at an estimated density of 1000 larvae per tank (58 larvae/l). Water flow into each tank was maintained at about 52 ml/second ($\pm$8.7, n=67). Larval feeding, sampling, and preservation methods were identical to Experiment 1.

Experiment 3. Striped bass eggs were spawned in a circular tank from one female in the presence of four males. At age 4.5 days post-hatch, larvae were transferred at a density of 19/l to 85-l glass aquariums equipped with the standpipe design described above. Larval feeding, sampling, and preservation methods were identical to the previous

experiments.

Experiment 4. In 1986 a special study was conducted on two-day old larvae to determine the effects of pH and aluminum on the skin. Groups of 65 striped bass larvae from the Weldon hatchery were kept in 2-l aquariums filled with filtered tap water. The experimental design consisted of a four by four matrix of pH (7.5, 6.5, 6.0, and 5.5) and concentrations of aluminum (0, 202, 405, and 608 ug Al^{3+}/l) as $AlCl_3$. High larval mortality was observed after 20 hours. The test was stopped and samples of the surviving larvae were fixed in a solution of 1.25% gluteraldehyde and 2% paraformaldehyde in a phosphate buffer (0.75 M) at pH 7.2. After dehydration in graded ethanol solutions, larvae were prepared for light microscopy or for scanning electron microscopy (SEM).

Histological Preparation

Striped bass larvae from Experiment 3 were prepared for histological examination in accordance with standard laboratory methods for paraffin processing. Fish larvae were placed on lens paper, which was folded to form an envelope, and placed in a plastic cassette. The cassette was either placed directly into a Fish Histomatic tissue processor in 10% buffered-neutral formalin, or stored in preservative for later processing. Larvae in the tissue processor were subjected to a 12-hour normal cycle paraffin infiltration process consisting of six separate baths of isopropyl alcohol ranging in concentration from 70%-100%, three xylene baths, and two paraffin infiltration baths. The infiltrated specimens were removed from the lens paper, oriented on agar blocks, and then placed into a cassette pan. Liquid paraffin was poured over the specimen to form a paraffin block.

Specimens were sectioned serially at 6-um intervals and floated in a water bath containing granular gelatin and an Aerosol solution to reduce surface tension. The resultant section ribbons were floated onto glass slides, which were then dried in an oven for 30 minutes at 60°C and allowed to cool to room temperature. The slides were stained with hematoxylin and eosin.

Wild striped bass larvae collected from the lower Roanoke River in 1984, 1985, and 1986 were also prepared for histological examination, but using a plastic embedding technique to minimize separation of tissues during sectioning. Larvae were removed from the formalin preservative and subjected to two 70% ethanol baths for one hour each, then two 100% ethanol baths for one hour each. No cassette was used in this procedure. Larvae were infiltrated with L.R. White plastic resin over a 24-hour period. Infiltrated larvae were then oriented in J.B.-4 molds; resin was added and polymerized in an oven at 60°C for 16-20 hours. Blocks were trimmed and mounted on a "00" size block for sectioning using a L.K.B. Cambridge Huxley microtome. Sections were floated in a 60% acetone-40% water bath onto slides and dried on a slide warmer. Specimens were stained with Toluidine Blue (1%) in a 1% sodium borate solution for 15 seconds at 60oC.

Specimens from the pH-aluminum experiment were post-fixed in a phosphate-buffered 1% OsO_4 solution, dehydrated in graded ethanol, critical-point dried, and coated with gold-palladium for examination under a ISO.40 Scanning Electron Microscope.

RESULTS

Larval Growth

Extensive mortality of the larvae was observed in experiments 1 and 2, which caused both experiments to end prematurely. Larvae were approximately 5 mm TL two days after hatch, and grew about 0.3 mm by day three (Table 1, Table 2). Growth averaged 0.2 mm from day three to day four. None of the larvae appeared to be in feeding condition. In both experiments, high mortality was probably caused by excessive turbidity during water release episodes from Roanoke Rapids Lake. Water quality remained fairly stable for both experiments (Table 3). Water temperatures ranged between 20°C and 24°C during the eight-day period.

Significant differences ($p = 0.05$) in rate of growth were observed between fed and unfed larvae in experiment 3. Fed larvae grew from 5.04 mm TL two days after hatching to 6.40 mm TL 11.5 days after hatching (Table

Table 1. Results of rearing experiment 1 showing age-length (±SD) relationship of striped bass larvae raised in ambient Roanoke River water. h.a.h. = hours after hatching; d.a.h. = days after hatching.

Date	Experimental Day	Sample Time	Age at sampling (h.a.h)	Age at sampling (d.a.h.)	Temp. (°C)	Mean length (mm TL) Fed 1*	Fed 3	Fed 5	Fed 8	Starved 2	Starved 4	Starved 6	Starved 7
4/23/85	1	1530	51.5	2	21.5 ±0.5 (2)	-	5.10 ±0.18 (12)	5.00 ±0.00 (9)	5.02 ±0.06 (10)	5.10 ±0.19 (8)	5.00 ±0.29 (7)	5.03 ±0.08 (12)	5.05 ±0.08 (10)
4/24/85	2	1200	72.0	3	20.0 ±1.0 (2)	-	5.44 ±0.18 (8)	5.44 ±0.30 (9)	5.45 ±0.27 (11)	5.43 ±0.30 (9)	5.48 ±0.32 (11)	5.36 ±0.32 (11)	5.30 ±0.19 (8)
4/25/85	3	1230	96.5	4	21.4 ±1.1 (3)	-	5.57 ±0.30 (8)	5.40 ±0.42 (5)	5.58 ±0.38 (6)	5.78 ±0.23 (9)	5.77 ±0.29 (13)	5.73 ±0.24 (11)	5.56 ±0.38 (5)
4/26/85	4	1200	120.0	5	23.1 ±1.1 (3)	5.80 (1)	-	-	-	5.50 (1)	-	6.1 (1)	5.5 (1)

*Refers to the number of the holding tank.

Table 2. Results of rearing experiment 2 showing age-length (±SD) relationship of striped bass larvae raised in ambient Roanoke River water. h.a.h. = hours after hatching; d.a.h. = days after hatching.

Date	Experimental Day	Sample Time	Age at sampling (h.a.h.)	Age at sampling (d.a.h.)	Temp. (°C)	Mean length (mm TL) Fed 1*	Fed 3	Fed 5	Fed 8	Starved 2	Starved 4	Starved 6	Starved 7
4/26/85	1	1700	48.0	2	23.1 ±1.1 (3)	5.00 ±0.00 (4)	5.00 ±0.00 (4)	5.00 ±0.00 (4)	5.00 ±0.00 (5)	5.25 ±0.5 (4)	4.82 ±0.24 (4)	5.10 ±0.22 (5)	4.90 ±0.14 (4)
4/27/85	2	1750	73.0	3	21.2 ±0.6 (3)	5.36 ±0.20 (8)	5.34 ±0.24 (9)	5.34 ±0.21 (7)	5.18 ±0.29 (9)	5.26 ±0.25 (9)	5.28 ±0.20 (9)	5.35 ±0.23 (8)	5.43 ±0.14 (9)
4/28/85	3	1830	97.5	4	23.2 ±1.0 (3)	-	5.83 ±0.58 (3)	-	5.70 ±0.24 (6)	5.50 (1)	5.50 ±0.00 (2)	-	5.56 ±0.43 (5)
4/29/85	4	1825	121.5	5	20.9 ±1.0 (3)	-	-	-	5.87 ±0.19 (7)	-	-	-	-
4/30/85	5	1630	143.5	6	20.8 ±1.5 (3)	-	-	-	6.00 (1)	-	-	-	-

*Refers to the number of the holding tank.

Table 3. Water quality information for the striped bass hatchery at Weldon, North Carolina, and the Roanoke River at Keeter's Dock (adjacent to hatchery) during the 1985 study. Dissolved oxygen (mg/l) and pH determined by Hach kit; nitrogen and phosphorus measurements (mg/l) by EPA methods. Total Al (ug/l) for unfiltered water samples. Unless noted, concentrations of Cr, Cu, Hg, Ni, Pb, and Zn (ug/l) below detectable limits*.

Date	Station	Time	pH	D.O.	T (^{o}C)	NH3-N	TKN	NO_2-N+ NO_3-N	PO4-P	Total P	Total Al	Other
4/23/85	Hatchery	1911	-	-	21.0	0.04	0.4	0.12	0.04	0.07	200	
	River	1930	-	-	-	0.06	0.2	0.17	0.04	0.07	200	
4/24/85	Hatchery	1730	7.5	10.0	21.0	0.02	0.4	0.11	0.04	0.06	200	
	River	1750	7.5	9.0	21.0	0.03	0.3	0.12	0.05	0.08	800	
4/25/85	Hatchery	1320	-	8.5	21.5	0.02	0.2	0.13	0.03	0.10	300	
	Hatchery	1845	7.5	9.3	22.5	0.03	0.5	0.13	0.04	0.07	300	Zn(30)
	River	1905	7.5	9.7	22.0	0.04	0.4	0.11	0.03	0.10	2400	
4/26/85	Hatchery	1700	7.5	9.2	24.0	0.05	0.3	0.09	0.05	0.08	200	
	River	1721	7.5	7.9	23.5	0.05	0.4	0.09	0.04	0.08	500	
4/27/85	Hatchery	1810	7.0	8.4	21.5	0.05	0.3	0.12	0.04	0.07	200	
	River	1820	7.0	8.1	21.0	0.08	0.4	0.13	0.04	0.07	400	
4/28/85	River	2030	-	-	-	0.09	0.4	0.16	0.04	0.10	800	
4/29/85	Hatchery	1800	7.5	8.5	21.5	0.11	0.4	0.20	0.06	0.09	200	
	River	-	7.0	9.4	21.5	0.13	0.4	0.22	0.07	0.10	300	
4/30/85	Hatchery	1658	7.8	9.0	22.5	0.06	0.3	0.20	0.05	0.09	200	
	River	1709	7.5	9.5	22.0	-	-	-	-	-	200	
5/1/85	Hatchery	1736	7.5	8.4	22.5	0.05	0.3	0.20	0.05	0.08	200	
	River	1746	7.5	9.3	22.5	0.04	0.3	0.20	0.05	0.09	600	

Table 3. (Continued)

Date	Station	Time	pH	D.O.	T (^oC)	NH3-N	TKN	NO_2-N+ NO_3-N	PO_4-P	Total P	Total Al	Other
5/2/86	Hatchery	1609	7.0	7.8	22.5	0.05	0.3	0.17	0.03	0.08	400	
	River	1603	7.0	8.2	22.5	0.05	0.3	0.16	0.02	0.12	1600	Pb(zoo)
5/3/85	Hatchery	1715	7.0	8.4	20.0	0.22	0.4	0.13	0.03	0.10	800	
	River	1730	7.0	8.1	20.0	0.21	0.3	0.14	0.04	0.11	1100	
5/4/85	Hatchery	1700	7.5	7.2	22.5	0.05	0.3	0.16	0.03	0.08	300	
	River	1730	8.0	9.4	22.0	0.06	0.3	0.18	0.04	0.08	700	Zn(20)
5/5/85	Hatchery	-	7.5	9.3	23.0	0.06	0.5	0.19	0.04	0.07	300	Zn(40)
	River	-	8.5	9.6	22.0	0.07	0.3	0.20	0.06	0.08	500	
5/6/85	Hatchery	1705	7.5	8.4	24.8	0.05	0.4	0.15	0.03	0.06	700	
	River	1715	7.5	8.8	23.2	0.06	0.3	0.14	0.03	0.11	1600	

*Minimum detectable limits (ug/l): Al=100; CR=50; Cu=20; Hg=0.2; Ni=100; Pb=100; Zn=20.

4), for an increase of 1.36 mm. Larvae were observed feeding actively on Artemia from 5 days post-hatch until the experiment terminated. Unfed larvae exhibited very little growth, averaging 4.93 mm TL on the fifth day after hatching and 4.98 mm TL by day 10, a change of less than 0.1 mm (Table 4). Significant difference in total length between the two groups was evident at 7.5 days post-hatch ($F=2.73$; $df=19, 14$; $P<0.05$). Water temperatures were between 20°C and 23°C during the 11-day period. Ammonia (NH_3-N) and total aluminum were the only water quality parameters that changed during experiment 3 (Table 3).

Histological Variation of Hatchery Larvae

The organs and tissues examined using light miscroscopy were: eyes, brain, gills, heart, liver, digestive tract, pancreas, kidney, trunk musculature, skin, and cartilage. A summary of the information is presented in Table 5. Comments on the degree of development are noted in the text where appropriate. Relative position of the organs and tissues of a Roanoke striped bass larva approximately eight days post-hatch are shown in Figure 2.

Eyes

Eye lens development was apparent in the youngest larvae examined (108 hours after hatching) and eye development appeared normal in both fed and starved larvae 6.5 days after hatching. By 7.5 post-hatch, abnormal development of the eyes was evident in several starved specimens. This abnormality appeared as reduced pigmentation in the retina (Figure 3). Sparce retinal pigment was also evident in unfed specimens 10.5 days and 11.5 days post-hatch.

Brain

Development of the brain appeared normal in all specimens.

Table 4. Results of rearing experiment 3 showing the feeding schedule, age at sampling, and mean length at sampling (mm TL) of fed and starved striped bass larvae raised in ambient Roanoke River water h.a.h. = hours after hatching ; d.a.h. = days after hatching.

Date	Experimental Day	Feeding (h.a.h.)	Age at Sampling (h.a.h.)	Age at Sampling (d.a.h.)	Mean length (TL) at sampling: Fed n	Fed mm	Starved n	Starved mm	T(°C) ± SD (n)	D.O. (mg/l) ± SD (n)
4/29/85	4	104	Stocked @ 19.5 larvae/liter						20.9	8.7
		106							±0.98	±0.66
		111.5	108	4.5					(3)	(3)
4/30/85	5	128.0							20.8	8.6
		131.5							±1.53	±0.45
		134.5	135	5.5	19	5.04	19	4.93	(3)	(3)
5/1/85	6	152.5							22.0	8.3
		158.5	159	6.5	19	5.23	19	4.98	±1.32	±0.40
									(3)	(3)
5/2/85	7	176.5							22.1	8.1
		180.0							±0.69	±0.52
		181.25	183	7.5	20	5.22	14	4.98	(3)	(3)
5/3/85	8	200.5							20.0	8.0
		203.0							±0.00	±0.35
		206.0	207	8.5	15	5.63	20	4.94	(3)	(3)
5/4/85	9	224.25							21.5	8.9
		228.0							±1.32	±0.46
		229.0	231	9.5	20	5.72	15	4.90	(3)	(3)

Table 4. (Continued)

Date	Experimental Day	Feeding (h.a.h.)	Age at Sampling (h.a.h.)	Age at Sampling (d.a.h.)	Mean length (TL) at sampling: Fed n	Fed mm	Starved n	Starved mm	T(°C) ± SD (n)	D.O. (mg/l) ± SD (n)
5/5/85	10	248.0							21.2	8.9
		251.5							±2.02	±0.53
		254.0	255	10.5	21	5.68	15	4.98	(3)	(3)
5/6/85	11	272.5							22.9	8.3
		274.5							±1.90	±0.17
		277.0	280	11.5	18	6.40	1*	2.60	(3)	(3)

*Remainder of larvae were contorted and could not be measured.

Table 5. Summary of the nutritional state of fed and unfed striped bass larvae raised in ambient Roanoke River water in 1985.

Tissue	Fed	Unfed
Nervous System		
Eye	Normal	Reduced retinal pigment in older larvae
Brain	no differences noted	
Notochord	no differences noted	
Respiratory System		
Gills	no differences noted	
Cardiovascular System		
Heart	no differences noted	
Gastrointestinal System		
Liver	large, well developed; glycogen accumulation	small, atrophied; nuclei closely spaced; no glycogen
Digestive Tract	partially or fully distended; food visible	collapsed; empty
Pancreas	islet of Langerhans well-developed; surrounded by exocrine pancreatic tissue	islet smaller; possible degeneration noted in one older specimen
Urinary System		
Kidney	hemopoetic tissue present in the cephalic kidney	hemopoetic tissue reduced in the cephalic kidney
Musculoskeletal System		
Musculature	robust, thickened; well developed	thin and fragmentary in older larvae
Cartilage	no differences noted	
Integumentary System		
Skin	no differences noted	

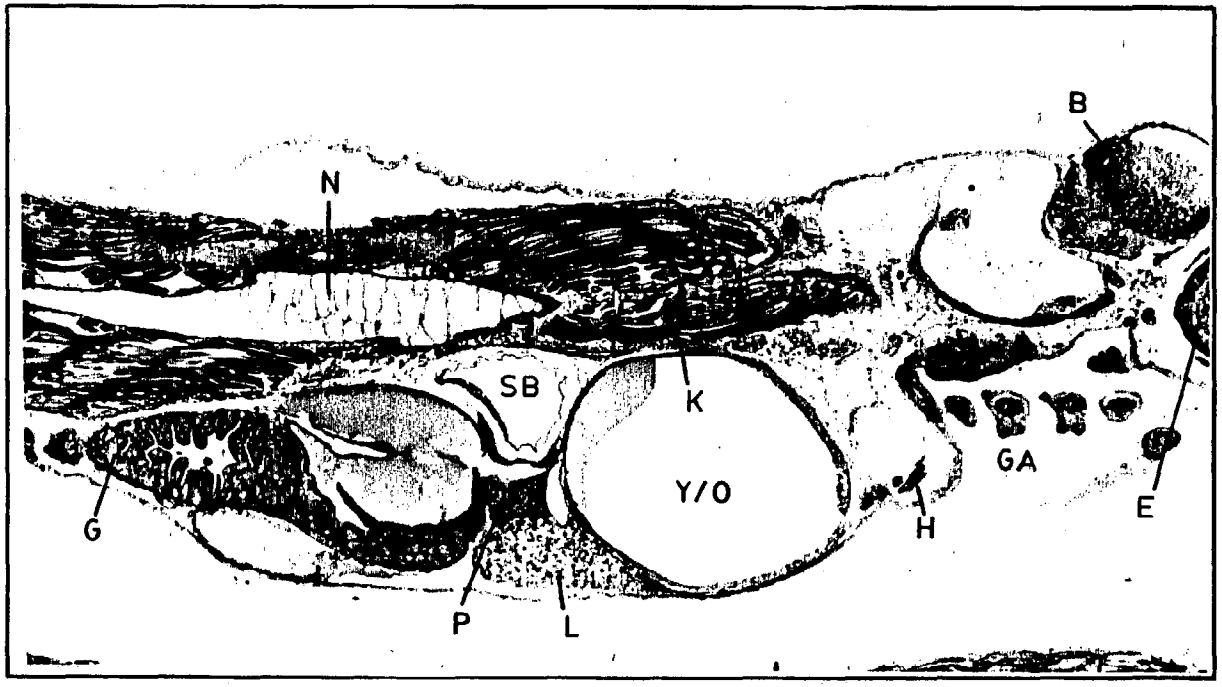
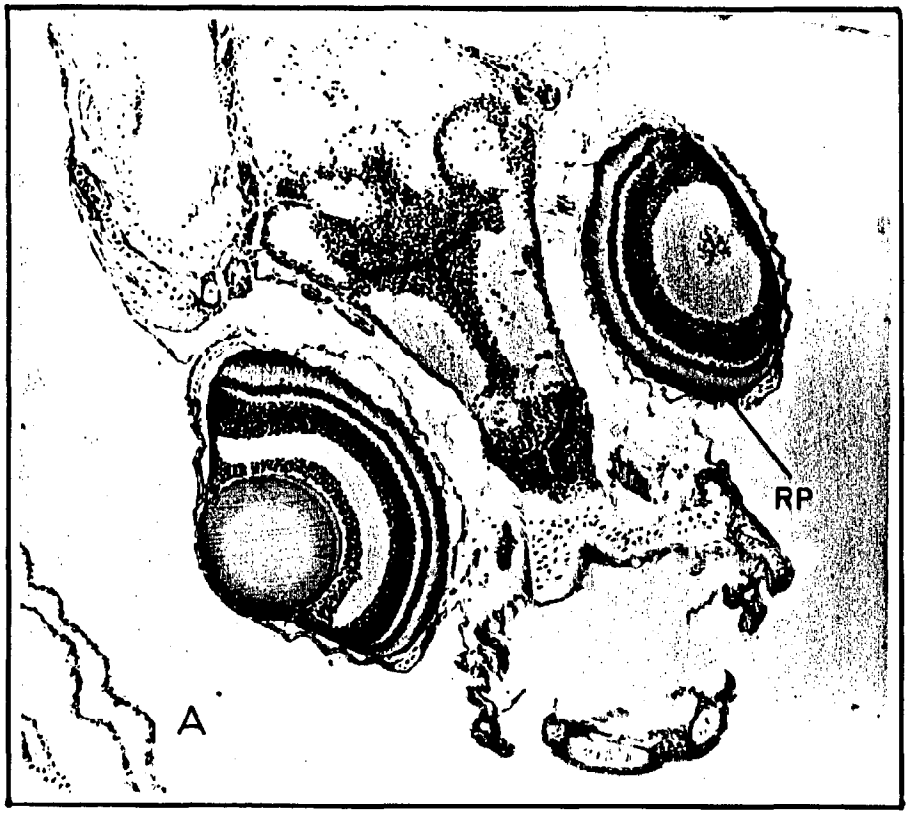


Figure 2. Sagittal section of a wild Roanoke striped bass larva (20x) approximately eight days post-hatch (6.5 mm TL) showing relative position of organs and tissues. G = gut; N = notochord; P = pancreas; L = liver; SB = swim bladder; K = kidney; Y/O = yolksac and oil globule; H = heart; GA = gill arch; B = brain; E = eye.



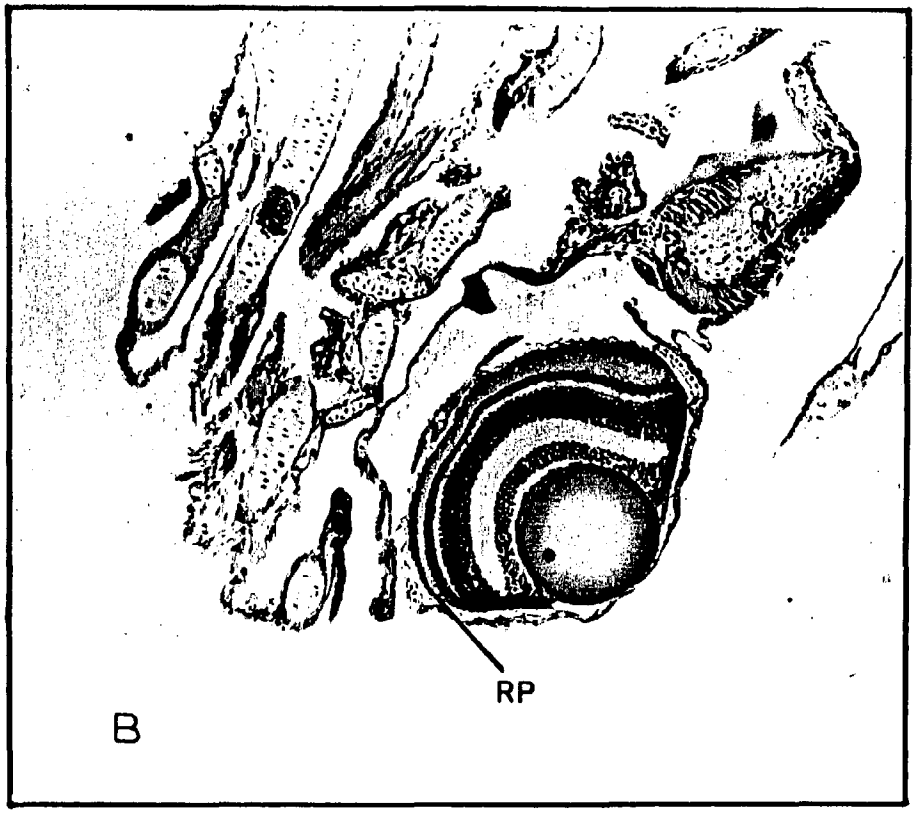


Figure 3. Eyes of striped bass larvae (50x) showing: (A) normal development of retinal pigment (RP) in fed specimens (7.5 days post-hatch); (B) reduced retinal pigment in unfed specimens (7.5 days post-hatch); and (C) eye degeneration in unfed specimens (11.5 days post-hatch).

Figure 3. (Continued)

Notochord

Development of the notochord appeared similar for both fed and unfed larvae. No shrinkage was evident.

Gills

Branchial arches were developing at 4.5 days post-hatch. Gill filaments were not developed, indicating that the gills were not functional at this stage of larval development. By 11.5 days after hatching, fed larvae possessed gill capillaries on the branchial arches (Figure 4). Gill development of unfed specimens appeared to be similar to fed specimens of the same age.

Heart

Both fed and unfed larvae had hearts that appeared as simple tubes 4.5 days after hatching. Development of the heart appeared normal in all specimens.

Liver

Livers of both fed and unfed larvae had mild to moderate multifocal glycogen accumulation, a condition normal for larvae still possessing yolk. Fed and unfed larvae possessed yolk 4.5 days after hatching; the rate of yolk absorption was highly variable in both treatment groups. By day 6.5, the livers of fed larvae possessed large amounts of glycogen. Unfed larvae had livers with compact cells, indicating reduction in glycogen content (Figure 5). Only fed larvae retained liver glycogen through day 12. Blood vessels containing red blood cells were visible in livers of fed larvae 10.5 days post-hatch.

Figure 4. Branchial arches of a fed striped bass larva showing: (A) arch configuration (20x); and (B) gill filament development (100x) at 11.5 days post-hatch.

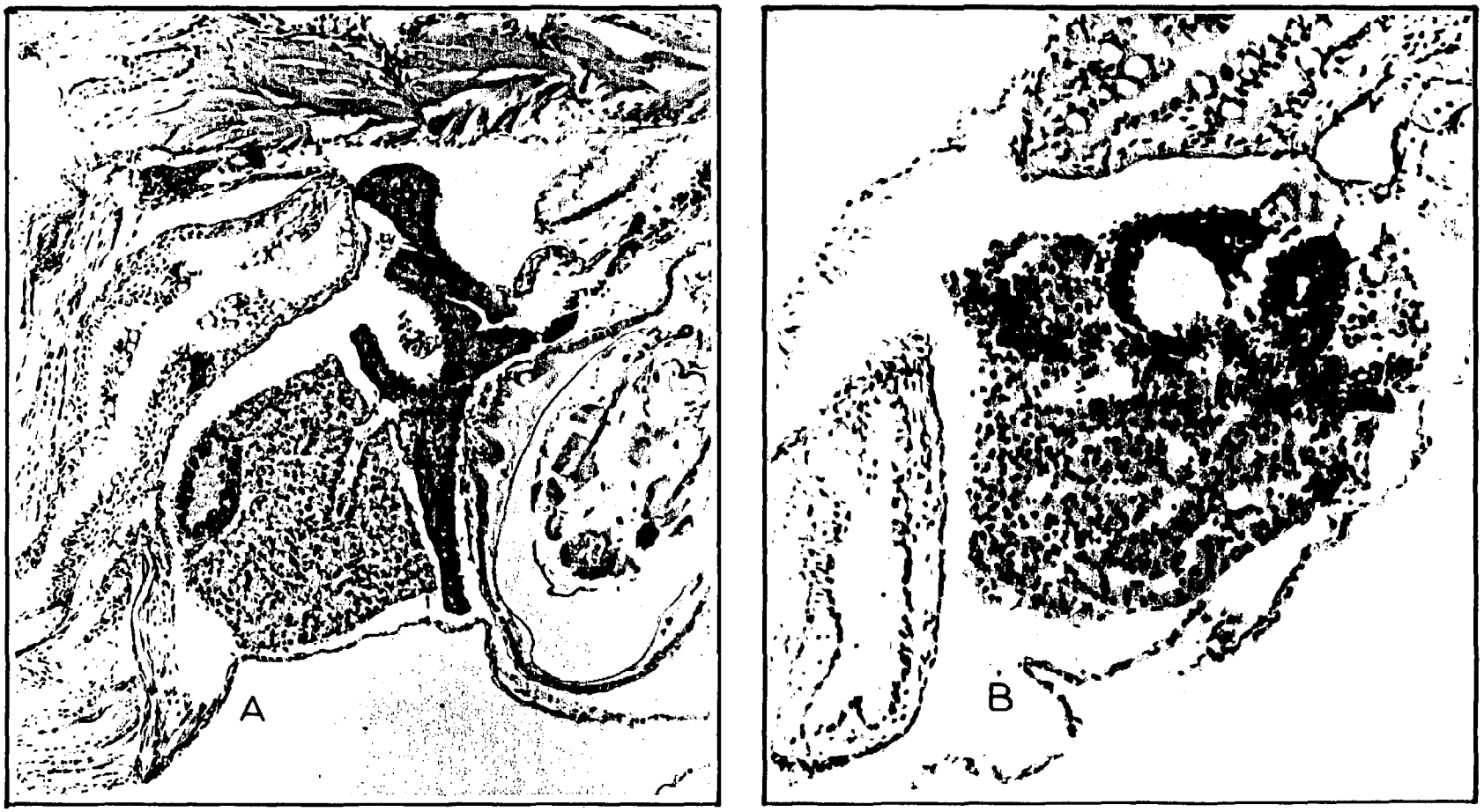


Figure 5. Liver of striped bass larvae showing: (A) glycogen (clear spaces) within the cytoplasm (50x) of a fed larva 12 days post-hatch; and (B) small compact cells with no glycogen (100x) of an unfed larva 10.5 days post-hatch.

Digestive Tract

The esophagus, midgut and hindgut of 4.5 day old larvae were well differentiated. No further differentiation was noted during the experimental period. Larvae that were fed Artemia exhibited partially distended guts after 6.5 days, indicating that feeding efforts were successful. All fed larvae possessed distended digestive tracts through day 11 (Figure 6). The guts of unfed larvae were collapsed and empty from 4.5 days post-hatch (Figure 6) through termination of the experiment; however, no degeneration of intestinal mucosa was observed.

Pancreas

At 4.5 days after hatching the pancreas was developed and appeared normal. By day 6.5, the islet (Isle of Langerhans) was visible surrounded by exocrine pancreas (Figure 7). The islet appeared to be slightly smaller in an unfed specimen 5.5 days old; the islet was not visible on histological sections of older larvae so effects of starvation could not be determined. No other differences in pancreatic development were observed between fed and unfed larvae.

Kidney

Pronephric kidneys appeared in larvae 4.5 days post-hatch, with few tubules extending cephalically to the liver region. By 12 days post-hatch, the kidney duct was visible extending caudally behind the rectum and hemopoietic (red blood cell forming) tissue was present cephalically and around the pronephric tubules (Figure 8). Hemopoietic tissue was reduced in unfed larvae 5.5 days post-hatch and older.

Muscle

Fed larvae possessed thickened, well-developed muscle fibers throughout the experiment (Figure 6). Muscle fibers appeared thinner and

Figure 6. Digestive tract (50x) of: (A) a fed larva 7.5 days post-hatch; and (B) an unfed larva 4.5 days post-hatch.

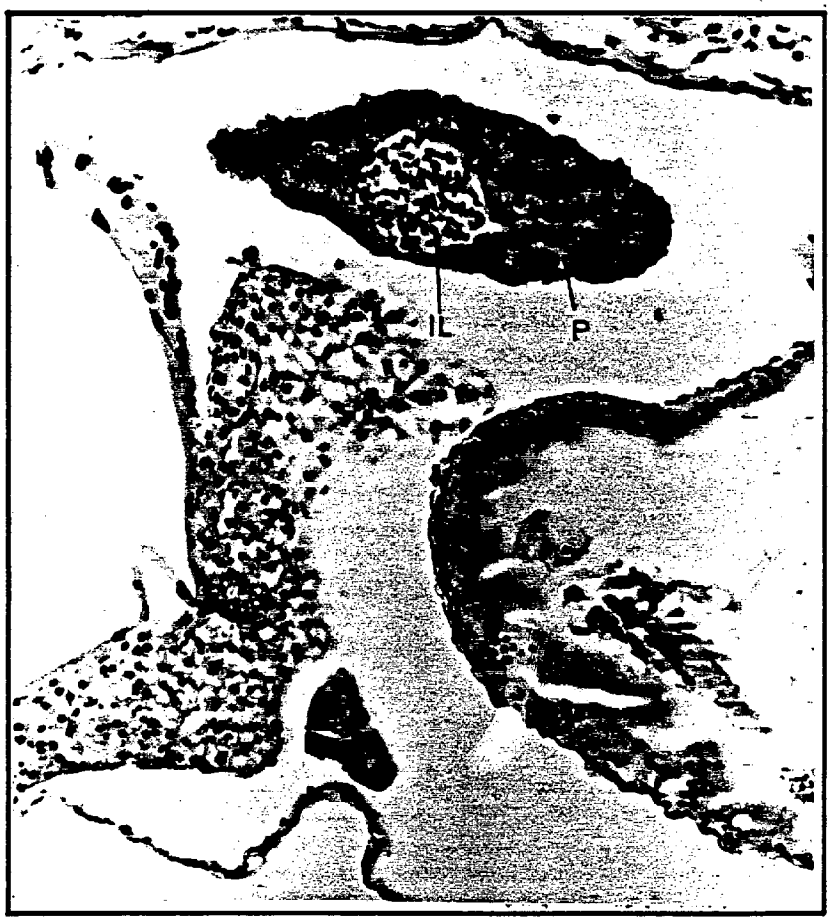


Figure 7. Pancreas (P) of a fed larva (100x) 6.5 days post-hatch with islet of Langerhans (IL) visible.

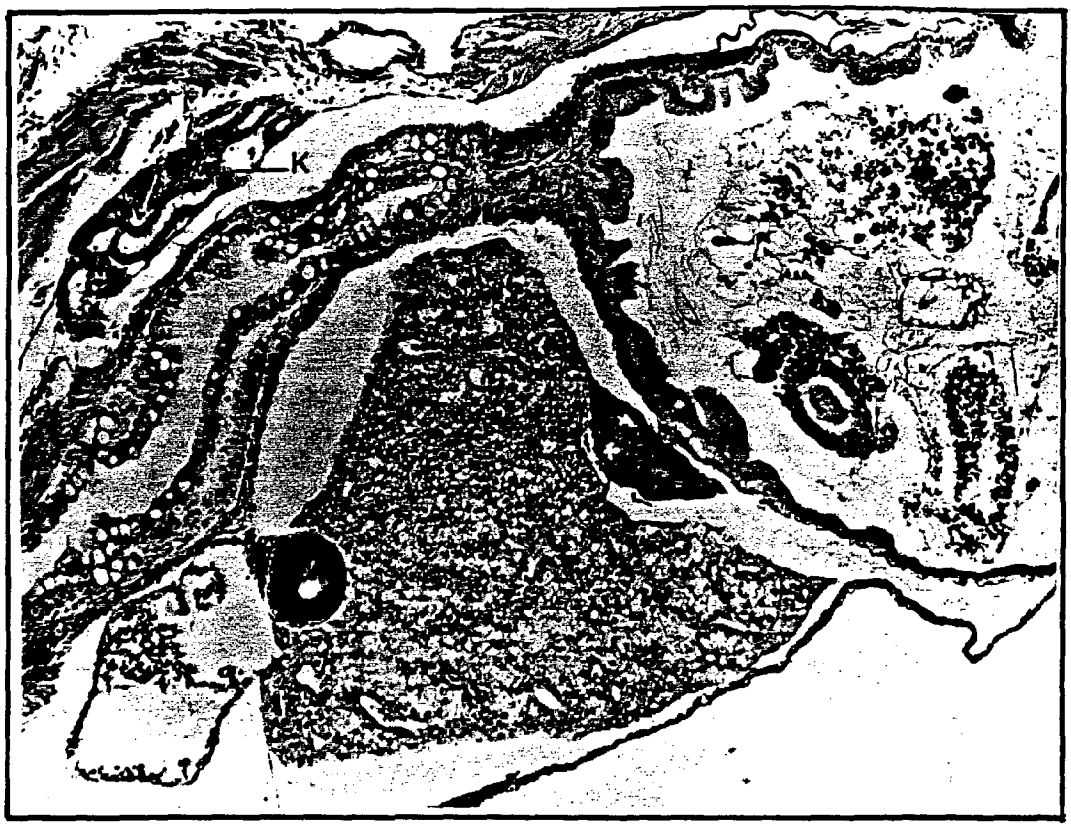


Figure 8. Pronephric kidney (K) of a fed striped bass larva (50x) 12 days post-hatch.

separated in unfed larvae as early as 6 days post-hatch. Older unfed larvae were difficult to section and came apart during processing, probably due to their frail condition. Fragmentation of muscle fibers was evident in unfed larvae 12 days after hatching, the same condition was noted by O'Connell (1976) for severely starved anchovy (Engraulis mordax) larvae.

Cartilage

No differences in cartilage development were noted between fed and unfed larvae. In the older larvae, cartilage was well developed. Cartilage cells of larval striped bass were in a wide extracellular cartilage matrix and similar in appearance to those of jack mackerel Trachurus symmetricus larvae (Theilacker 1978) and starved northern anchovy larvae (O'Connell 1976).

Skin

Cross-sections of the epidermal layers showed no visible differences between fed and starved larvae. By 10.5 days post-hatch, scale-producing cells were visible in the epidermis. Tooth development was visible in the oral cavity of fed larvae 11.5 days after hatching.

Histological Examination of Wild Larvae

Histological examination showed normal tissue development in 60 striped bass larvae collected from the lower Roanoke River in 1984, 1985 and 1986 (Table 6). Food items were present in the digestive tracts of several specimens, indicating successful feeding. The empty digestive tracts of the other wild larvae were deeply invaginated and had thicker walls than unfed larvae from experiment 3, indicating that the larvae were not in a starved condition. All specimens had large cells in the esophagus, and livers ranged from mostly dense and compact to moderate glycogen accumulation. Several larvae had abnormal body proportions (i.e., stunted in appearance), but appeared normal histologically. The stunted

Table 6. Capture date and location of histologically-examined wild striped bass larvae collected from the lower Roanoke River and western Albemarle Sound in 1984-1986. Station numbers as in Figure 1.

Date	Station	Number examined	Remarks
5/22/84	1	4	Normal
	11	1	"
5/23/84	3	3	"
	10	3	"
5/29/84	9	3	"
	13	1	"
	15	2	Stunted appearance; histologically no difference compared to lab-fed larvae
6/2/84	13	1	Normal
6/8/84	13	7	"
4/28/85	8	3	"
	13	3	"
5/16/85	4	2	"
5/22/85	6	3	"
	11	5	"
5/24/85	6	2	"
5/26/85	8	2	Cross-sections of cladocerans in gut
6/2/85	6	3	Normal
6/6/85	9	3	"
5/22/86	11	4	"
5/31/86	6	5	"

larvae still possessed yolk and not all organs were fully developed. Therefore, the stunted appearance was probably not due to lack of food.

Aluminum-pH Effects on Skin

Light microscopy observations of larval striped bass skin exposed to low pH in the presence of aluminum showed no clear morphological differences among the experimental groups. However, observations using scanning electron microscopy showed clear differences in the surface pattern of epidermal cells between control larvae and larvae exposed to the various concentrations of pH and aluminum. Larvae exposed to high aluminum concentrations showed a clear reduction of epidermal microridges on the head and caudal fin (Figures 9-11).

DISCUSSION

Histological comparisons indicate that fed and unfed striped bass larvae show physiological differences: starvation is apparent as either development retardation or tissue degeneration as early as 5.5 days post-hatch. The results of our study are similar to those reported by O'Connell (1976) for early post yolk sac larvae of northern anchovy (*Engraulis mordax*). In our study, the organs and tissues showing signs of poor nutritional state included the eye, liver, digestive tract, kidney, and musculature. The pancreas may also be an indicator organ. These changes occurred within 11 days post-hatch. Theilacker (1978) found that larval jack mackeral (*Trachurus symmetricus*) show the first signs of poor nutritional state in the digestive tract and associated glands and the extent of cellular deterioration increased with length of starvation. Martin and Malloy (1981), using slightly larger (and presumably older) striped bass larvae than we used, found that starvation effects could be detected in four tissues: abdominal body wall, epaxial musculature, gut epithelium, and liver. Their study suggested that retinal tissue may be the most sensitive to poor nutritional state, but indicated that additional studies were needed. Research conducted at the University of Rhode Island

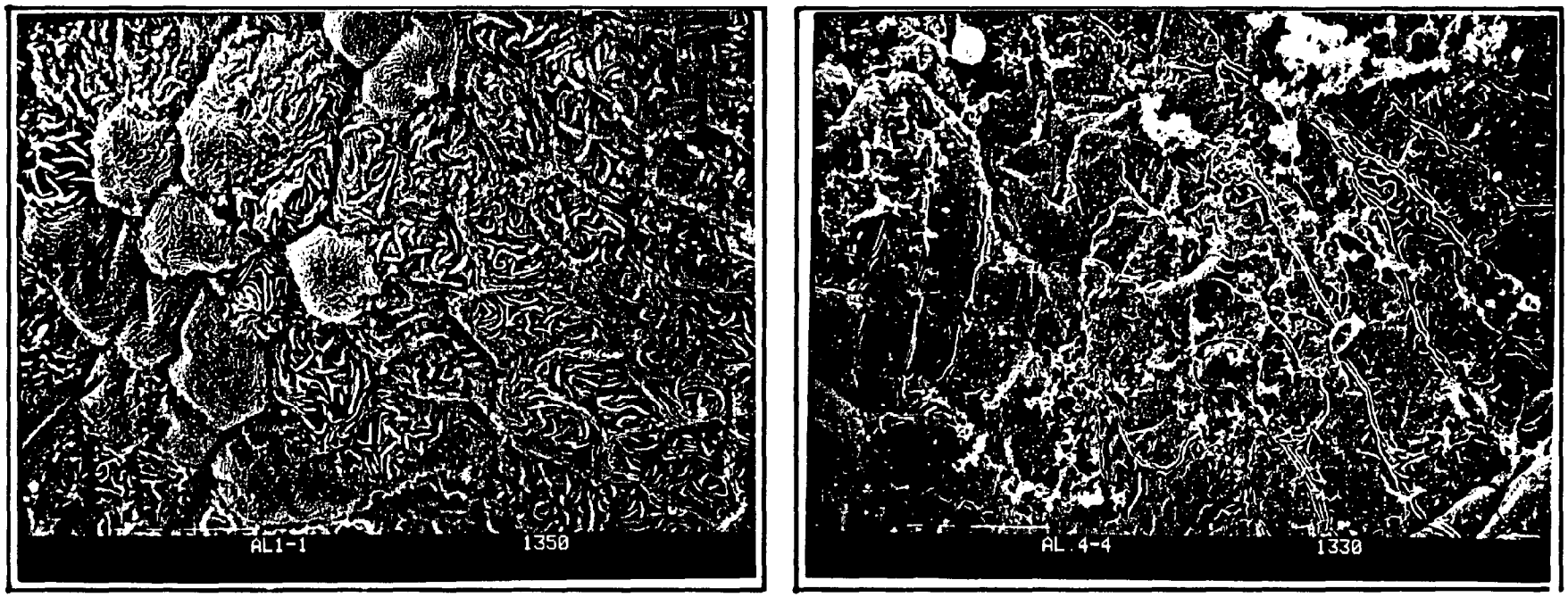


Figure 9. Scanning Electron Microscope (SEM) micrographs (1350x) of the epidermis in the head region of striped bass larvae held for 20 hours: (A) in tap water at pH 7.5; and (B) in water containing 608 ug Al^{3+}/l (22.5 uM) at pH 5.5.

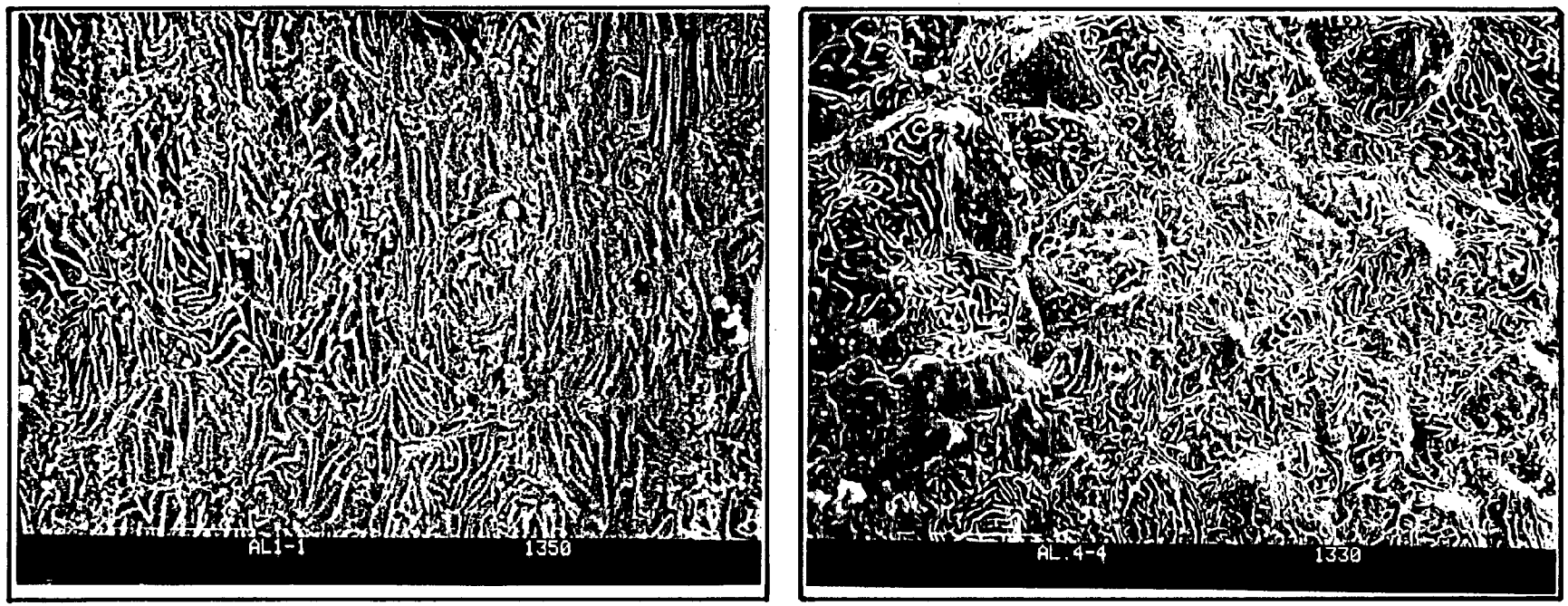


Figure 10. Scanning Electron Microscope (SEM) micrographs (1350x) of the epidermis in the bell region of striped bass larvae held for 20 hours: (A) in tap water at pH 7.5; and (B) in water containing 608 ug Al^{3+}/l (22.5 uM) at pH 5.5.

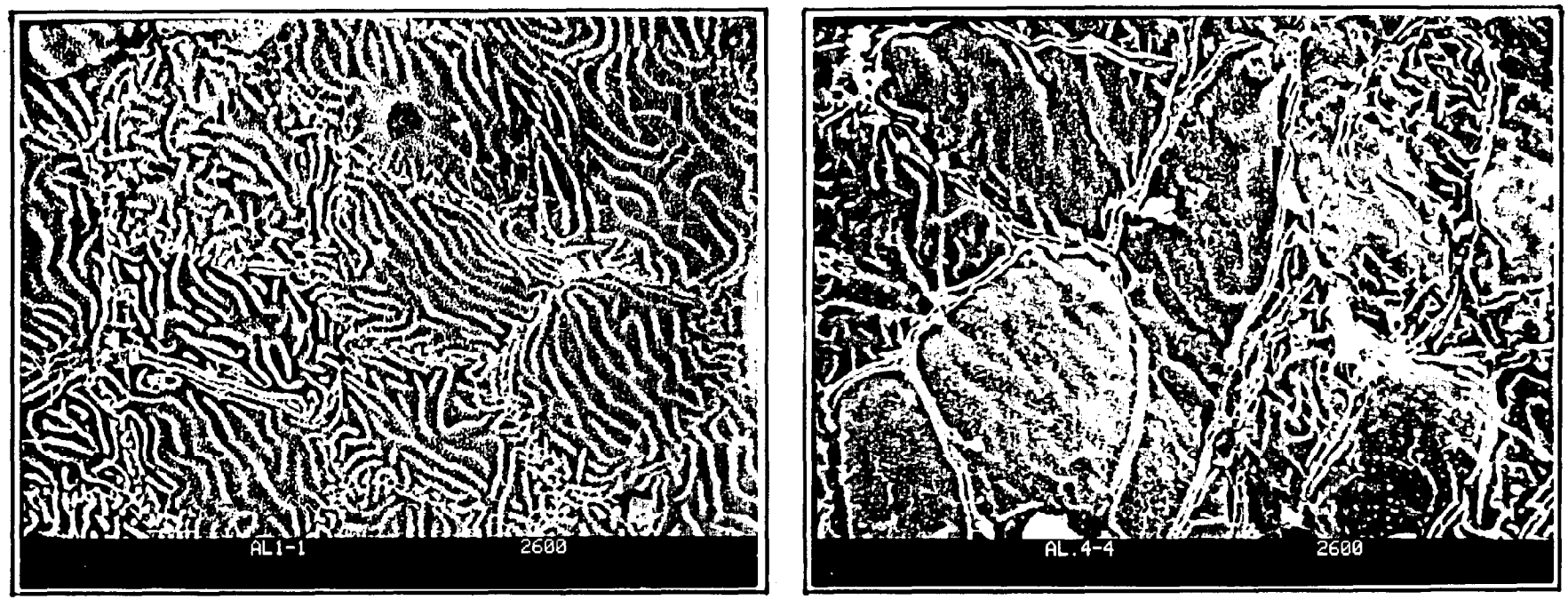


Figure 11. Scanning Electron Microscope (SEM) micrographs (2600x) of the caudal fin epidermis of striped bass larvae held for 20 hours: (A) in tap water at pH 7.5; and (B) in water containing 608 ug Al^{3+}/l (22.5 uM) at pH 5.5.

on the eye and optic nerve of striped bass larvae indicate degeneration of the retinal tissue and optic nerve within several days after last feeding (Joel Bodammer, URI, 1985, personal communication). None of these studies examined effects of starvation on skin.

Changes in the morphological characteristics or external appearance of fish larvae have been used as diagnostic tools to identify starvation, but this technique may not be applicable for striped bass several days post-hatch. Shelbourne (1957) assessed the nutritional state of Pleuronectes platessa larvae based on their external appearance. Morphological changes used as diagnostic characters include decreased thickness in body of the larvae (Kostomarova 1962; Nakai et al. 1969; Arthur 1976; Ehrlich et al. 1976; Powell and Chester 1985; Grover and Olla 1986), and changes in fin angle and the ratio of head to eye height (Ehrlich et al. 1976). Theilacker (1978) used a combination of five morphometric measurements (standard length, head length, eye diameter, body depth at pectoral, and body depth at anus) to estimate the nutritional state of jack mackeral larvae; similar results were obtained for spot (Leiostomus xanthurus) by Powell and Chester (1985). Grover and Olla (1986) found significant differences in seven of eight morphometric measurements of "starved" and "normal" sablefish (Anoplopoma fimbria); nutritional state was made circumstantially by analysis of gut contents. Martin and Malloy (1981) found that head depth, body depth at pectoral, and the ratio of body depth at anus to head depth could be used to identify starving striped bass larvae reared in the laboratory; however, they recommended further study to relate these changes to wild larvae.

Stunted wild striped bass larvae were present in small numbers in Roanoke River samples, especially in 1984, but stunting may not have been caused by starvation. Koo and Johnston (1978) reported that eggs of striped bass exposed to sudden temperature changes resulted in deformed larvae. Deformities appeared as shortened bodies, enlarge finfolds, and curved or twisted spines. In our larboratory experiments, the only noticeable morphometric differences of striped bass larvae were in the body lengths of fed (longer) and unfed (shorter) larvae. O'Connell's (1976) experiment used anchovy larvae similar in age and size to the larval

striped bass reared in our experiment 3. His growth studies showed significant differences in larval body length as starvation progressed. Decreased mean length with age was assumed to represent shrinkage, which is known to occur in starved herring larvae after yolk absorption (Blaxter and Hempel 1963). O'Connell (1976) concluded that none of his morphological measurements including larval length could be used to diagnose the starving condition of northern anchovy larvae for the first nine days of life, but might be useful for older larvae. Since the stunted wild striped bass collected from the Roanoke River were still in the yolk stage, we can speculate that stunting was caused not by starvation but by environmental factors such as sudden changes in temperature or pH.

Evidence of poor nutritional state was not observed in the 60 wild larvae examined, but this information does not contradict the possibility that a high incidence of starvation occurs in the Roanoke River. It is quite likely that larvae in a starving condition are easily preyed upon or are not susceptible to capture by our nets as they cannot maintain equilibrium in the water column, thereby causing them to sink. This statement is supported by the fact that in 1984, during high flow conditions, larvae that had died prior to collection comprised 10-50% of all larvae in the samples (Rulifson et al. 1986). This phenomenon was not observed in low flow years (1985 and 1986), suggesting that reduced river turbulence minimizes net capture of dead or dying larvae.

Larval starvation is probably one of the principal causes of mortality (Hunter 1976), and is hypothesized as one of the contributors to poor year classes of Potomac striped bass between 1974 and 1977 (Martin and Malloy 1981). Results of our study indicate that striped bass larvae, if unsuccessful at feeding while absorbing the yolk, will undergo physiological changes that may further reduce their chances of feeding successfully after the yolk is absorbed. Thus, the chances of postyolk larvae feeding successfully are reduced further as starvation progresses: visual contact with prey organisms will be reduced due to changes or reduction in retinal pigment; degenerative trunk musculature and poor glycogen reserves reduce the chances of successful feeding strikes; blood-forming tissues are reduced, resulting in reduced capacity of the blood to

provide a nutrient supply to tissues; reduced growth limits the ability of the larvae to select prey of a nutritionally valuable size; and larvae weakened from starvation are more susceptible to predation and unfavorable environmental conditions.

Larvae must encounter an abundant food source when feeding is initiated to optimize feeding success and increase chances of survival after the yolk is absorbed. The timing of this encounter is dependent upon several factors: (1) location of egg deposition by striped bass adults; (2) speed of larval transport downstream; (3) effect of water temperature on larval growth; and (4) seasonal timing of striped bass spawning to coincide with maximum zooplankton production. In most watersheds supporting anadromous striped bass populations, such as those in Chesapeake Bay tributaries, these four factors coincide for the most part without human intervention. Such is not the case in the Roanoke River watershed, which is dominated by reservoirs for recreation and hydropower generation. Water flow is determined by planned water releases that may not reflect natural seasonal changes in river flow. Zooplankton concentration during the low flow years are highest in the Roanoke River delta (Rulifson et al. 1986), primarily in the Middle (Station 9) and Cashie Rivers (Stations 8 and 11) near the Highway 45 bridge (Figure 1). Few zooplankton are present in western Albemarle Sound at the same time (Rulifson 1984; Rulifson, Stanley and Cooper, unpublished data). Therefore, under spring conditions of low flow (as in 1985), striped bass larvae must begin to feed in the Roanoke River delta where zooplankton concentrations are highest in order to optimize changes of feeding successfully.

High-flow conditions were predominant in 1984, resulting in the appearance of striped bass eggs and yolksac larvae in western Albemarle Sound. Zooplankton concentration in this area was very low compared to that in the delta. The mean abundance of eggs and larvae were significantly correlated with water discharge from Roanoke Rapids Lake lagged by three days; these life stages were swept downstream into the delta from upstream areas in three days (Rulifson et al. 1986).

The effect of water releases in large quantities for hydropower generation will affect striped bass larvae in several ways. First, the

temperature of released water is cooler than the water downstream. This acts to curtail spawning activity of the adults and slows the development of the larvae. Second, the spawning location (distance upstream) is selected by striped bass adults based upon the amount of water flowing downstream which determines water depth in spawning areas. This strategy ensures the necessary length of river and flow rate needed at a particular water temperature to optimize the number of eggs hatching successfully, and subsequent development of yolksac larvae to the feeding stage. Sudden changes (increases or decreases) in water flow after eggs are spawned causes an imbalance of this delicate set of conditions, resulting in high egg and larval mortality. Third, high flow conditions are not conducive for zooplankton production: turbulence, turbidity, and high rate of downstream transport do not allow the zooplankton community to reach the concentration needed for successful feeding of striped bass larvae.

Predictions can be made concerning the length of time needed (depending on river flow) from the spawning location of the eggs to the food supply for larvae in the Roanoke River. Information needed for these predictions include: (1) estimated spawning location; (2) time required for eggs to hatch; (3) time after hatch at which feeding begins; and (4) speed of travel from spawning grounds (as an egg) to optimum foraging grounds (as a larva). For purposes of argument, we assume that Halifax (river mile 120) is the primary spawning ground for adult striped bass. Rearing experiments show that striped bass eggs held in ambient Roanoke River water hatch in approximately 48 hours from spawning. Larval feeding begins 5.5 days after hatching, or about 7 days after they were spawned. Significant differences ($P<0.05$) in growth of fed and unfed larvae are apparent 7.5 days after hatching (10.5 days after spawning); larvae feeding successfully are about 5.5 mm TL at this time (Table 4). Therefore, larvae must begin to feed between 7 and 9 days after they are spawned.

The last component of information needed to complete the scenario is missing: no information is available on the relationship between flow rate measured at Roanoke Rapids and time of travel downstream. An additional unknown quantity is whether this relationship remains linear over the distance traveled.

However, we do have two years of information on the abundance of larvae and zooplankton under conditions of high flow (1984) and low flow (1985). In 1984, transport of eggs and larvae downstream took only three days under a flow regime of a mean daily rate of 14,000-18,000 cfs. Larval abundance showed no correlation with low flows (2,000-6,000 cfs) in 1985. Therefore, the best flow regime for optimizing the nutritional state of striped bass larvae is between 2,000 cfs and 14,000-18,000 cfs; it must also be the flow rate that transports larvae to the delta 7 to 9 days after they are spawned at Halifax.

Results of the 1984 and 1985 Roanoke River field studies are depicted in Figure 12. In these simplified drawings, the importance of being in the right place at the right time becomes apparent. In 1984, Stage 1 larvae (those still possessing yolk) exhibited peak abundance upstream of peak zooplankton concentrations. Therefore, the chances for successful feeding at this crucial stage of development were quite low. By the time the fish absorbed the yolk (Stage 2 larvae), they were past the greatest zooplankton concentrations (Figure 12) and chances of feeding successfully were poor. Gut analyses of 1984 Stage 1 and 2 larvae indicated that the rate of successful feeding was poor. This must have resulted in high larval mortality, because the juvenile trawl index for 1984 was 0.0 fish/trawl (N.C. Div. Mar. Fish.), the worst on record. In 1985, conditions were more favorable for larval feeding. The abundance of Stage 1 and Stage 2 larvae occurred downstream close to peak zooplankton abundance (Figure 12). The number of both Stage 1 and Stage 2 larvae with food in their guts was much higher than in 1984, and the juvenile trawl index of 0.32 young-of-year/trawl (N.C. Div. Mar. Fish.) indicated better survival.

Trauma to the skin may also contribute to mortality of striped bass larvae. Since the gills and other body organs are not completely developed and functional at early larval stages, the skin functions as the major system in ion exchange and osmoregulation. Disease organisms such as fungi, bacteria, and parasites present in the water have easy access to skin. One adaptation to these stresses is in the specialized surface structure known as microridges (Yamada 1968).

Examination of the skin using scanning electron microscopy indicated

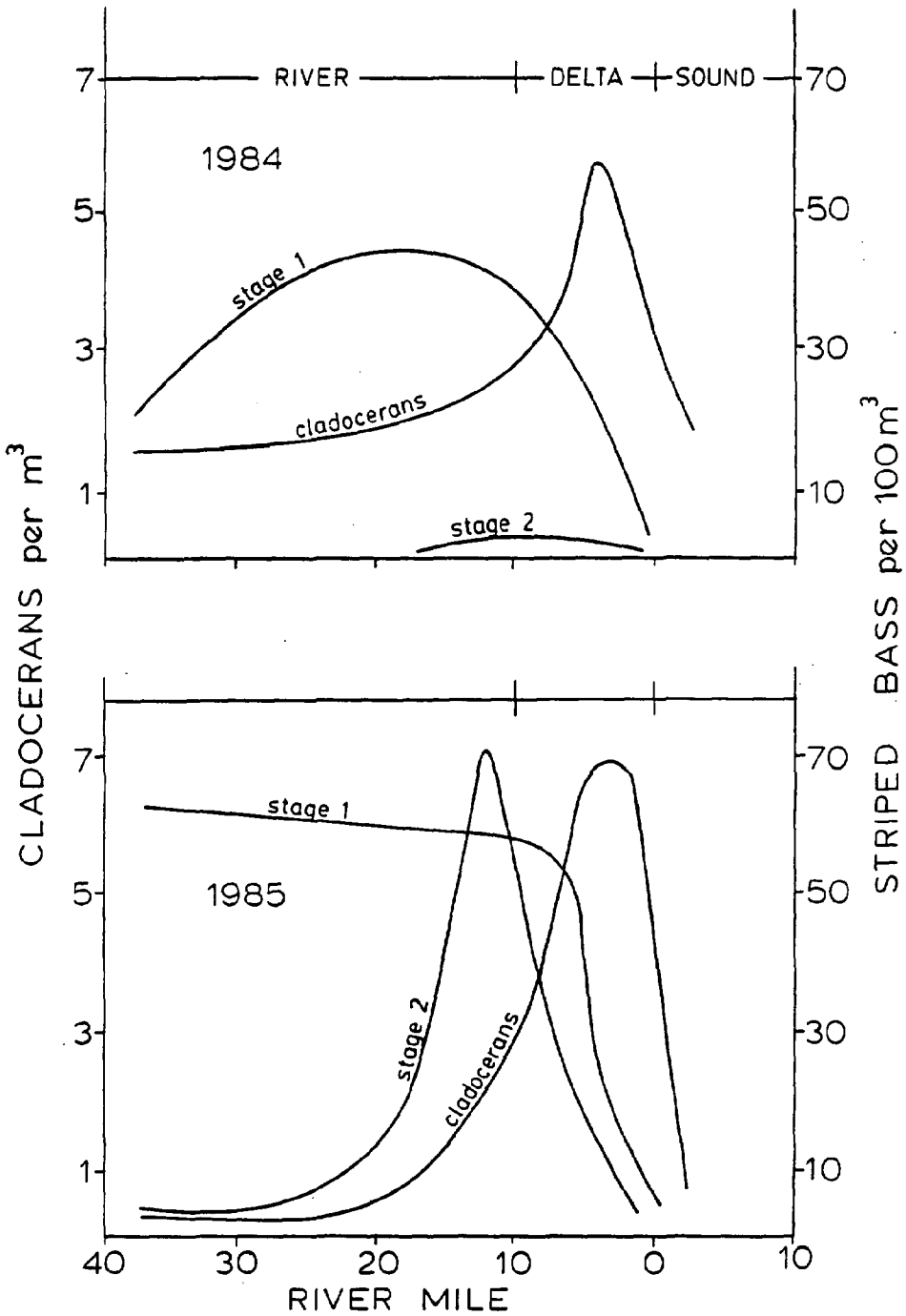


Figure 12. Simplified drawing depicting the springtime densities of Stage 1 (with yolk) striped bass larvae, Stage 2 (no yolk) larvae, and zooplankton in the Roanoke River, North Carolina, in a year of high river flow (1984) and low river flow (1985).

changes in the microridge structure after striped bass larvae had been exposed to low pH (5.5) and high concentrations of aluminum (608 ug Al^{3+}/l). These conditions are lethal to striped bass larvae in the laboratory (Mehrle et al. 1984; Colombo, unpublished data) and are suspected of contributing to mortality of larvae held under *in situ* conditions in the Nanticoke River estuary (Hall et al. 1985). Klauda and Palmer (1986) reported abnormalities in the integument of blueback herring (*Alosa aestivalis*) yolk-sac larvae exposed to low pH and high aluminum. Total aluminum values as high as 2400 ug/l were recorded in the Roanoke River during our study (Table 3). The methodology used to determine pH did not have the accuracy required for data comparisons with other studies. However, our results suggest that striped bass larvae may exhibit high mortality one to two days after hatching due to skin stress, followed by a second mortality due to starvation.

SUMMARY

Striped bass larvae held in ambient Roanoke River water were feeding successfully on *Artemia* by 6.5 days post-hatch. The growth of unfed larvae was significantly less ($P<0.05$) than fed larvae at 7.5 days post-hatch. Histological examination showed a decline in the nutritional state of unfed larvae as soon as 5.5 days after hatching. By 11.5 days post-hatch, unfed larvae showed abnormal eye development, reduction in liver glycogen, collapsed and empty digestive tracts, reduced hemopoietic tissue in the pronephric kidneys, and thin and separated muscle fibers. The 60 wild Roanoke River larvae histologically examined showed no signs of starvation. We believe that larvae dying from or weakened by starvation are easily preyed upon or are not susceptible to net capture. High concentrations of aluminum (608 ug Al^{3+}/l) in low pH waters (5.5) alters the microridge structure of larval skin, which functions as the gill at this stage of development. Poor water quality may place undue stress on the skin, resulting in high mortality one to two days post-hatch. Another peak in mortality may occur from starvation effects 5-10 days post-hatch.

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