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Effects of environmental factors on survival, growth, and production of American shad larvae

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Episodic increases in temperature of $\geq 5°$ C above 20° C, over 48 h or declines in pH of 1·0 unit from pH 7·0 reduced survival of yolk-sac and feeding-stage larvae of American shad *Alosa sapidissima*. Over 16 days all measures of survival, growth, and production were more favourable at each higher temperature in the 15–25° C range. More favourable responses were also obtained at the higher prey level (500 *v.* 50 *Artemia* nauplii l^{-1}) and at the higher pH (7·5 *v.* 6·5). Combinations of high temperature and high prey levels, at pH 7·5, led to highest larval production. Little growth or production occurred at 15° C, regardless of pH or prey level. The effect of pH was strong with respect to survival, but weak with respect to growth. In attempts to restore American shad populations by larval stocking, release times and sites can be critical to optimize survival and eventual returns. Releases of larvae potentially will be most effective when made at temperatures >20° C, pH>7·0, and prey levels >50 l^{-1}. These conditions are most likely to occur in Maryland tributaries of Chesapeake Bay between mid-May and early June.

Key words: shad; larvae; survival; growth; environmental factors.

INTRODUCTION

American shad *Alosa sapidissima* Wilson stocks have declined throughout Chesapeake Bay (U.S.A.) and its tributaries during the past century (Foerster & Reagan, 1997). The most recent declines, which have occurred since the 1970s, border on collapse. Historically, major declines in this anadromous species were attributable to damming of tributaries, which rendered many spawning grounds in Chesapeake watersheds inaccessible to returning adults. Historical and recent heavy fishing in Chesapeake Bay, its tributaries, and coast-wide is also believed to have played a significant role in the decline of this highly sought commercial and recreational species (CBP, 1989). American shad fishing presently is under moratorium in Chesapeake Bay and the prospects for recovery of the stocks are unclear. Restoration efforts involve improved fish passage in tributaries, transportation of adult spawners to suitable spawning sites, and releases of hatchery-produced larvae and juveniles (St Pierre, unpubl.). The larval and juvenile releases promise to be effective means to restore American shad to several Bay tributaries and watersheds (Hendricks, 1995).

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Releases of young hatchery-produced American shad can be effective and larval marking and recapture–recognition methods are well developed (Hendricks *et al.*, 1991), but there has been relatively little evaluation of the role of environmental factors and their effect on young American shad survival or production. Year-class strength of American shad probably is determined by numbers that survive embryonic and larval stages (Crecco & Savoy, 1984), and survival during critical early life stages is correlated with environmental factors (Crecco *et al.*, 1983, 1986; Crecco & Savoy, 1984; Savoy & Crecco, 1988). Few field experiments, except for some to evaluate predation on released larvae (Johnson & Dropkin, 1992; Johnson & Ringler, 1995), have been carried out to determine optimum water quality, weather, or environmental conditions associated with successful stocking of American shad larvae and juveniles.

Eggs and larvae of American shad, like those of most teleosts, are sensitive to variation in environmental factors. Klauda *et al.* (1991) reviewed information on environmental requirements, noting that eggs and larvae were the most critical life stages. They reported that eggs required temperatures >13° C, pH>6·0 and dissolved oxygen >5·0 mg l^{-1}, while larvae required temperatures in the range 15·5–26·1° C, pH>6·7, and dissolved oxygen >5·0 mg l^{-1}. Klauda (1994) presented results of experimental research on effects of acidification and metals on American shad eggs and larvae, demonstrating that pulsed inputs and 1–3-day exposures to low pH and high metal levels, especially aluminium, could cause extensive mortalities.

Experimental tests indicated that salinity, within the probable limits that American shad eggs and larvae would be exposed to in estuarine tributaries, was not potentially harmful (Limburg & Ross, 1995). However, Zydlewski & McCormick (1997) determined that American shad develop salinity tolerance at metamorphosis, between 26 and 45 days posthatch, and exposure to sea water (35 psu) before 18 days posthatch caused 100% mortality. In the laboratory, growth and condition of American shad larvae at 20° C in tests of 6–9 days duration was related directly to prey levels (Johnson & Dropkin, 1995).

In nature, less is known of sensitivity of eggs or larvae to specific levels of environmental factors. Crecco & Savoy (1985, 1987*a*, *b*) found that growth and survival of American shad larvae in the Connecticut River were related directly to zooplankton densities and temperature, but related inversely to volume of river flow. In the Hudson River, Limburg (1996) reported that most juvenile pre-recruits had originated from spawning in late season (i.e. June) when river temperatures were increasing, river flows were declining, and zooplankton levels were high.

Published information suggests that American shad larvae have comparatively broad tolerance ranges to environmental factors, in accord with expectations that offspring of anadromous spawners should be adapted to withstand environmental fluctuations that are common in tributaries during spring months. In this respect, American shad are not unlike striped bass *Morone saxatilis* (Walbaum) eggs and larvae, which are spawned under similar conditions (Rutherford & Houde, 1995; Secor & Houde, 1995; Houde *et al.*, 1996). American shad eggs may be more sensitive than striped bass to pH fluctuations, especially in poorly buffered tributaries (Klauda *et al.*, 1991). There is little information to predict

how combined effects of temperature, prey levels, and pH may affect survival and production of American shad larvae.

The present study, tested the combined effects of temperature, prey levels, and pH on survival and growth of American shad larvae in laboratory experiments. It was hypothesized that combinations of low temperatures and pH levels, which occur seasonally in Chesapeake Bay tributaries, and low prey availability, would reduce survival potential of American shad larvae significantly. Interaction effects among these three factors were hypothesized to be potentially important. In addition, it was hypothesized that combined episodic drops (i.e shocks) in temperature and pH, which occur commonly in tributaries after storms during spring months, would cause episodic mortalities in yolk-sac and feeding-stage larvae, even when such temperature and pH declines taken alone might have little effect.

MATERIALS AND METHODS

EXPERIMENTS

Two types of experiment were run. In the first, pH and temperature shocks were administered to either yolk-sac or feeding-stage larvae. These experiments were of 48 h duration. In the second, long-term tests of 16-day duration evaluated combined effects of pH, temperature, and prey level on feeding-stage larvae that were 4–20 days old (Table I).

SOURCES OF LARVAE

Larvae were obtained from Manning Hatchery, Maryland Department of Natural Resources. Adult spawners were of Hudson River and Delaware River origin (Table I). Eggs or larvae for the experiments originated from spawns of multiple females and males. The exact number of adults which produced spawns is unknown. Incubation and rearing temperatures at the Manning Hatchery ranged from 17·0 to 19·0° C, and pH levels were >7·5. Eggs for shock experiments or yolk-sac larvae for the long-term experiment were transported from the hatchery to the Chesapeake Biological Laboratory (*c.* 1 h) in insulated boxes which contained 15-l plastic bags of oxygenated water.

GENERAL EXPERIMENTAL CONDITIONS

Experiments were run at the Chesapeake Biological Laboratory. Well water, adjusted to 0·5 psu salinity by addition of Patuxent River estuarine water, was the experimental medium. All tests were run in a temperature-controlled laboratory under 14L : 10D photoperiod conditions. Immersion heaters and water baths were used to control experimental temperatures. Monophosphate and dibasic phosphate buffers were used to control pH, and 0·6 M HCl or 0·6 M NaOH were added by pipette as needed to maintain designated pH levels. The larvae were fed newly hatched *Artemia* nauplii. Aliquots of nauplii at known concentrations were added periodically to treatments to maintain nominal prey levels.

EXPERIMENT 1; YOLK-SAC LARVAE SHOCKS

The experiment was initiated on 26 May 1995. Approximately 5000 newly hatched yolk-sac larvae were held for 36 h in an aerated 78-l aquarium at 20·0° C, pH 7·0, and conductivity 1110 µS (0·5 psu salinity) until the experiment began. A two-factor (five temperatures × three pH levels) experiment was run with duplicated treatments. All combinations of designated pH and temperature levels were tested (Table I). Thirty jars of 2-l capacity were set at 20° C and pH 7·0 initially. Twenty-five, 2-day-old larvae were stocked into each jar. Aeration and mixing were controlled in each jar by pumping air through a 1·5-mm diameter Pasteur pipette at a rate of one to two bubbles per s.

TABLE I. Summary of experiments to evaluate combined effects of temperature, pH, and prey level on survival, growth, and production of American shad larvae

Yolk-sac larvae shock	
Source of adult stock	Hudson River
Preshock	20° C, pH 7·0
Shock	20 control, 20→30, 20→25, 20→15, 20→10° C pH 7·0→6·0, 7·0→6·5, 7·0→7·5
Expt. duration	48 h
Age of larvae	2–4 days posthatch
No. of experimental jars	30
Tabulated results	Table II
Feeding-stage larvae shock	
Source of adult stock	Hudson River
Preshock	20° C, pH 7·5
Shock	20 control, 20→30, 20→25, 20→15, 20→10° C pH 7·0→6·0, 7·0→6·5, 7·0→7·5
Expt. duration	48 h
Age of larvae	6–8 days posthatch
No. of experimental jars	30
Tabulated results	Table II
Three-factor combination (long-term)	
Source of adult stock	Delaware River
Temperature	15, 20, 25°C
pH	6·5, 7·5
Prey level	50, 500 l^{-1} *Artemia* nauplii
Conductivity	mean=936, range=678–1291 μS
Expt. duration:	16 days
Age of larvae	4–20 days posthatch
No. of experimental tanks	24
Tabulated results	Table III

Temperature and pH shocks then were administered by adjusting temperature and pH to the designated shock levels (Table I) incrementally over 24 h. Treatments were maintained at the designated pH and temperature shock levels for an additional 24 h before being terminated. Survivors were counted and stored in ethanol.

EXPERIMENT 2: FEEDING-STAGE LARVAE SHOCKS

This experiment followed experiment 1 and utilized the same stock of American shad larvae. Larvae were held in the 78-l, aerated aquarium at 20·0° C and pH 7·0 until the experiment began on 30 May 1995. The two-factor (five temperatures × three pH levels) experiment was run with duplicated treatments. All combinations of designated pH and temperature levels were tested (Table I). The protocol for shock treatments was identical to that described for experiment 1, except that larvae were fed *Artemia* nauplii at a level of 50 l^{-1} in the experimental jars.

EXPERIMENT 3: THREE-FACTOR, LONG-TERM EXPERIMENT

Yolk-sac larvae, 3 days posthatch, were transported to the Chesapeake Biological Laboratory on 23 May 1995 and held in an aerated 78-l aquarium at 19·5° C and pH 7·0–7·4. The experiment was initiated on 24 May, when 100, 4-day posthatch larvae, were stocked into 39-l experimental tanks. Treatments were assigned randomly to the 24 aquaria, all of which were set at 20·0° C, pH 7·0 and 0·5 psu salinity initially, then adjusted gradually to the designated test levels (Table I) over a 48-h period. *Artemia*

nauplii were added to the tanks at designated treatment levels (50 or 500 l^{-1}) on the first day of the experiment. The 16-day experiment was a three-factor (three temperatures × two pH levels × two prey density levels) design. All possible combinations of temperature, pH, and prey density levels were included and assigned randomly in the duplicated experiment. To maintain water quality, 50% was replaced in each aquarium on every third day of the experiment. Temperatures were adjusted as required; pH levels were determined two to four times per day and adjusted if necessary; assigned prey levels were maintained throughout the 14-h lighted period each day after estimating prey concentrations from sampled aliquots three to five times per day and adding nauplii as needed to maintain assigned concentrations. Aeration and mixing were controlled in each aquarium by pumping air through a small aeration stone at a rate of *c.* five bubbles s^{-1}. Dead larvae were removed and counted daily. Survivors were counted at the termination of the experiment (20 days posthatch) and preserved in ethanol for future measuring and dry weighing.

ANALYSIS

Survival was the measured response variable in the two shock experiments. Survival, growth, and production responses were analysed in the long-term experiment. Two-factor (shock experiments) and three-factor (long-term experiment) analysis of variance (ANOVA) were applied to the data to determine if there were differences in mean responses among temperature, pH, or prey treatment levels. If the ANOVA result was significant, Tukey's-hsd or a least-squares means procedure was applied to identify specifically means that were significantly different. Where possible, interaction effects were evaluated in the analyses.

The ANOVA procedure was applied to per cent survival (arcsine transformed), standard length (mm) at termination of the experiment, length-specific growth rate (day^{-1}), dry weight (mg) at termination of the experiment, weight-specific growth rate (day^{-1}), gross production (mg dry weight), net production (mg dry weight), and coefficients of variation for standard lengths and dry weights. Log transformations were applied to data when necessary to help meet assumptions of ANOVA. Normality was tested by the Wilk–Shapiro statistic and homogeneity of variances by Bartlett's test and Welch ANOVA. In the analysis of survival for the feeding-stage larvae shock experiment and that for gross production in the long-term experiment, the assumption of normal distributions was not met. The assumption of homogeneity of variances, however, was met in all cases. Since the consequences of non-normality are not too serious for the test of significance (Sokal & Rohlf, 1969), the ANOVA was carried out for these responses.

Net production (P_{net}) is the difference in dry weight biomass between the termination and beginning of an experiment,

$$P_{net} = B_{t_1} - B_{t_0}$$

where:

B_{t_1} = biomass at termination of experiment,
B_{t_0} = biomass at beginning of experiment.

Gross production (P_{gross}) also accounts for growth of individuals that died subsequently before termination of the experiment (Ricker, 1978),

$$P_{gross} = G(B_t - B_0)/(G - Z)^{-1}$$

where:

G = weight-specific growth rate,
Z = instantaneous mortality.

In the long-term experiment, the trends in mortality among treatments were also compared in χ^2 tests of independence. Observed and expected mortalities were calculated

TABLE II. Yolk-sac and feeding-stage larvae shock experiments; analysis of variance probability levels for effects of temperature and pH on per cent survival (arcsine transformed)

Factor	Yolk-sac larvae	Feeding-stage larvae
Temperature	0·132	0·0001
pH	0·079	0·0001
Temp × pH	0·2801	0·0002
Overall ANOVA	0·1309	0·0001

for designated 4-day periods, and a χ^2 value computed. Observed mortalities were tallied from the daily collections of dead larvae in each experimental tank.

RESULTS

EXPERIMENT 1: YOLK-SAC LARVAE SHOCKS

The temperature and pH shocks did not cause significant effects (at $\alpha = 0{\cdot}05$) on the highly variable survival of yolk-sac larvae (Table II). However, the low mean survival rates suggested that episodic increases in temperature (from 20° to 30° C) and decreases in pH (7·0–6·0) reduced survival potential in these 48-h tests [Fig. 1(a)]. Overall, mean survival of yolk-sac larvae in this experiment was 13·2%.

EXPERIMENT 2: FEEDING-STAGE LARVAE SHOCKS

Temperature and pH shocks affected survivorship of the 6–8-day posthatch larvae strongly [Table II, Fig. 1(b)]. A pH shock of −1·0 unit, or a temperature shock of +10° C killed all larvae. A temperature shock of +5° C also reduced survival dramatically. The best overall survival occurred in treatment combinations in which pH was increased from 7·0–7·5. The temperature shock of 20–15° C resulted surprisingly in relatively high survival rates. In this experiment, the interactive effects of increasing pH and decreasing temperature yielded the highest survival [Fig. 1(c)]. Because a pH control, with pH held at 7·0, was not included, we cannot judge whether a higher survival response might have been associated with constant pH.

EXPERIMENT 3: THREE-FACTOR-LONG-TERM EXPERIMENT

Designated temperatures of 15, 20, or 25° C, pH levels of 6·5 or 7·5, and prey levels of 50 or 500 *Artemia* nauplii l^{-1} had major effects on the nine response variables (Table III). Analysis of variance results (Table IV) indicated that survival, growth, and production were impacted strongly. The significant interaction effects indicate that responses often were complex, especially for weight growth and production (Table IV). There was little indication of

FIG.

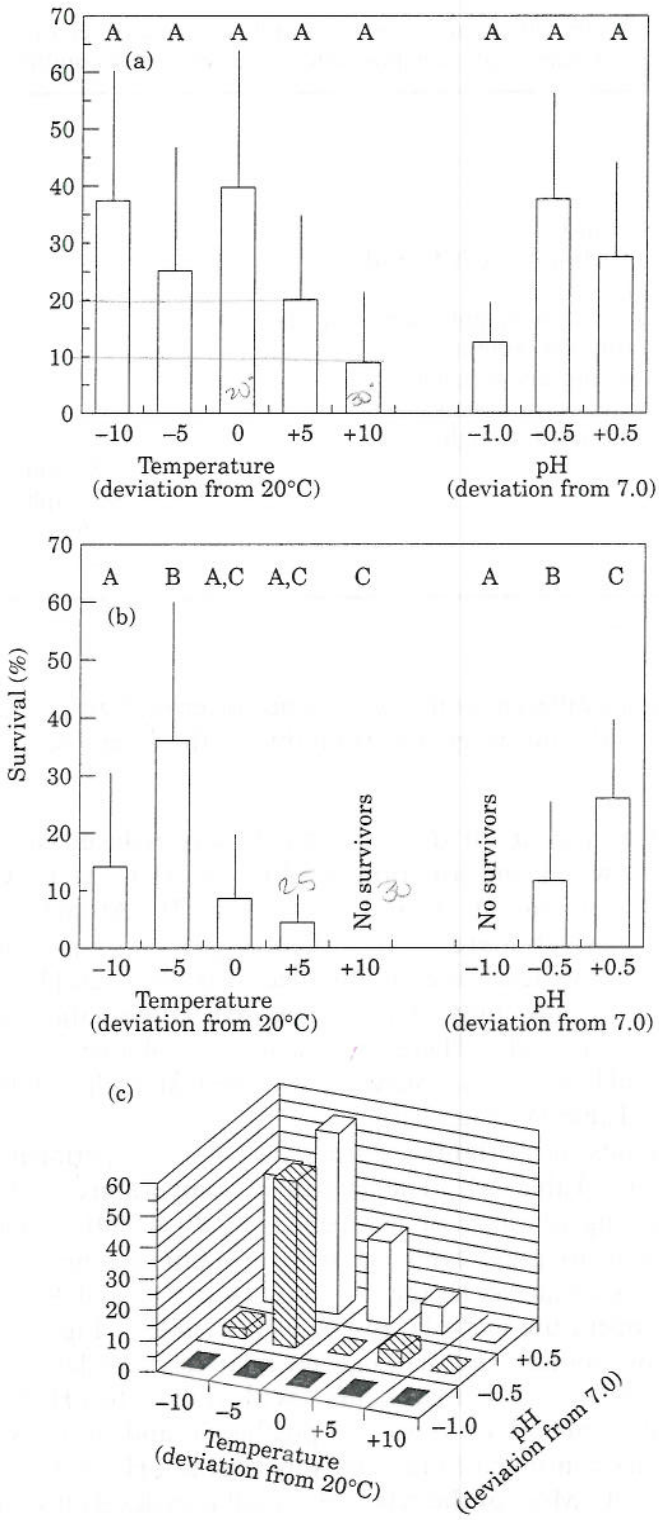

FIG. 1. American shad larvae. Mean survival, temperature and pH shock experiment. Shocks indicated as deviations from 20°C and pH 7·0 initial conditions. Error bars are +2 S.E. Different letters above bars indicate means that are significantly different ($P<0·05$) in analysis of mean arcsine-transformed percent survival for (a) yolk-sac larvae, (b) feeding-stage larvae, and (c) feeding-stage larvae, temperature × pH interaction effects.

TABLE III. Summary of mean responses and 1 standard error (in parentheses) of American shad larvae at 20 days posthatch in three-factor combination experiments

Response variable		Mean response
Survival (%)		18·45 (3·09)
Standard length (mm)		15·59 (0·34)
Instantaneous growth-in-length rate (day^{-1})		0·022 (0·001)
Dry weight (mg)		1·63 (0·23)
Instantaneous growth-in-weight coefficient (day^{-1})		0·121 (0·008)
Net production (mg dry weight)		15·50 (7·89)
Gross production (mg dry weight)		48·55 (9·92)
Coefficient of variation in length (%)		9·64 (0·69)
Coefficient of variation in weight (%)		36·62 (2·70)
Mortality	5–8 dph	19·2 (2·0)
	9–12 dph	48·5 (1·7)
	13–16 dph	27·5 (5·3)
	17–20 dph	15·9 (2·4)

dph, Days posthatch.

among-treatment differences in the variance of growth responses (coefficients of variation in length and weight) attributable to the three factors.

Survival

Survival of larvae at 20 days posthatch was reduced significantly at low temperatures, low pH and low prey level (Table IV). At 15° C, mean survival rate was <25% of that at 20 and 25° C (Fig. 2). At pH 6·5 and prey level $50\ l^{-1}$, mean survivals were *c.* 50% those at pH 7·5 or prey level $500\ l^{-1}$. A significant pH × prey level interaction effect was observed [Table IV, Fig. 3(a)], indicating that a low prey density–low pH combination was particularly detrimental to survival. There was also a probable interactive effect of temperature × pH ($P=0·06$). Survival was best at high pH–high temperature combinations [Table IV, Fig. 3(b)].

The time trends in mortalities during the 16-day experiment differed among treatment levels (Table V). The effects of temperature and pH levels were particularly strong (χ^2 tests of independence, $P<0·0001$) while weaker effects were attributable to prey levels ($P<0·05$). Major among-levels differences in daily mortalities occurred in the 5–8-days and 5–8 and 9–12-days posthatch periods for temperature and pH, respectively (Table 5, Fig. 4). Daily mortality at 15° C was low initially, but then became relatively high later in the experiment, in contrast to 20 and 25° C treatments. With respect to pH, highest mortalities were observed at pH 6·5 on days 5–8 posthatch, and on days 9–12 at pH 7·5. Daily mortalities continued to be relatively high at pH 6·5 during the last 8 days of the experiment. Most of the relatively small prey-level effect was attributed to higher mortalities at the low prey level in the initial 5–8-day posthatch period.

Sizes and growth

Mean lengths of larvae at termination of the experiments varied directly with increasing levels of temperature, pH and prey. The effects of prey level and

TABLE IV. Three-factor combination experiment, American shad larvae, 20 days posthatch, analysis of variance probability levels for effects of temperature, pH, and prey levels on nine response variables

Factor	Response variables								
	Survival[a]	Length	g	Dry wt[b]	G	P_{net}[c]	P_{gross}[d]	CV_{len}	CV_{wt}
Temperature	0·0001**	0·0001**	0·0002**	0·0001**	0·0001**	0·0001**	0·0001**	0·1698	0·2977
pH	0·0005**	0·0402*	0·0555	0·0005**	0·0017**	0·0001**	0·0001**	0·8301	0·5653
Prey level	0·0007**	0·0001**	0·0003**	0·0001**	0·0001**	0·0001**	0·0001**	0·5667	0·8038
Temp × pH	0·0589	0·1796	0·2024	0·0148*	0·0349*	0·0191*	0·0248*	0·2328	0·5239
Temp × prey	0·2213	0·0769	0·1438	0·0028**	0·0025*	0·9378	0·2115	0·0391*	0·4906
pH × prey	0·0140*	0·8663	0·8178	0·1562	0·2128	0·0006**	0·0140*	0·9497	0·6875
Temp × pH × prey	0·4222	—	—	—	—	0·7621	—	0·1989	0·5236
Overall ANOVA	0·0002**	0·0001**	0·0002**	0·0001**	0·0001**	0·0001**	0·0001**	0·1329	0·6541

g, Length-specific growth rate (mm day^{-1}); G, dry weight-specific growth rate (day^{-1}); P_{net}, net production; P_{gross}, gross production; CV_{len}, coefficient of variation in standard length; CV_{wt}, coefficient of variation in dry weight.

[a]Per cent survival, arcsine transformed; [b]$\log_{10}$ (dry weight); [c]$\log_{10}$ (P_{net} + 19·1). Because minimum value for $P_{net} = -18·1$, the log transformation was done on scaled values of P_{net} + 19·1; [d]$\log_{10}$ (P_{gross}).

*Significantly different F in GLM analysis ($P<0·05$).

**Highly significantly different F in GLM analysis ($P<0·01$).

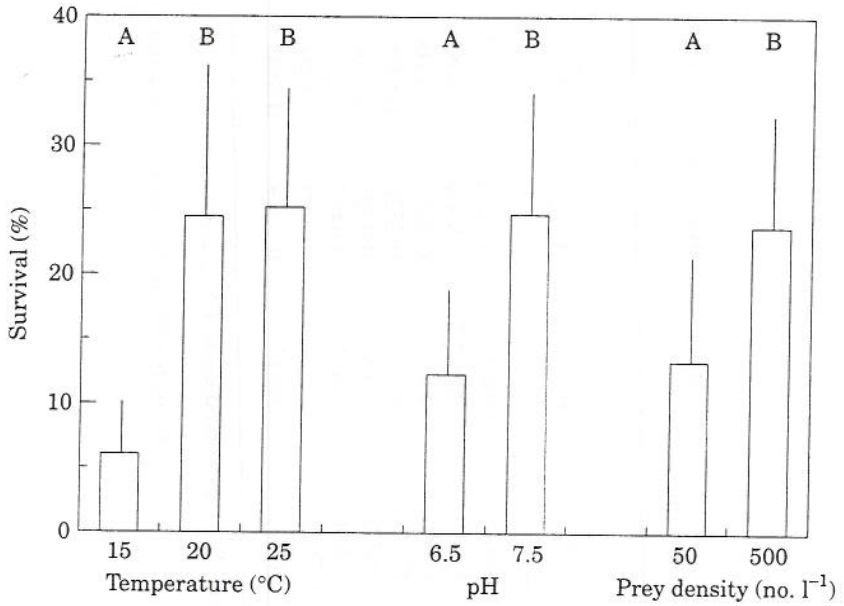

FIG. 2. American shad larvae. Three-factor combination (long-term) experiment, mean survival at 20 days posthatch. Error bars are +2 S.E. Different letters above bars indicate means that are significantly different ($P<0{\cdot}05$) in analysis of mean arcsine-transformed survival.

temperature were much stronger than that of pH (Table IV). Length-specific growth coefficients were significantly lower at the lowest temperature (15° C) and prey level ($50\,l^{-1}$) than at higher temperatures and prey (Table IV, Fig. 5). For mean lengths and length-specific growth coefficients, there were no significant interaction effects in the ANOVA analyses.

The effects of temperature, pH, and prey level on mean dry weights at 20 days posthatch and mean weight-specific growth coefficients were even more pronounced. Mean weights and coefficients varied directly and strongly with increasing temperature, pH, and prey levels (Table IV, Fig. 6). Effects of temperature × prey level interactions were strong, indicating that American shad larvae grew poorly at the low temperature (15° C), regardless of prey level, and grew in weight particularly fast at the high temperature–high prey level combination [Fig. 7(a)]. A weaker, but significant interaction effect of temperature × pH indicated that weight growth was best at the highest temperature, and that low pH had little negative influence on biomass growth of larvae when temperature was high (25° C) [Fig. 7(b)].

Production

Both net and gross productions increased steadily and significantly as temperatures, pH and prey levels increased (Table IV, Fig. 8). The significant temperature × pH interaction effect resulted from the relatively rapid increase in production as temperature increased when pH was 7·5 compared to pH 6·5 [Fig. 9(a)]. The significant pH by prey density interaction effect resulted because production was nearly independent of pH at the $500\,l^{-1}$ prey level but related directly to pH at the $50\,l^{-1}$ prey level [Fig. 9(b)].

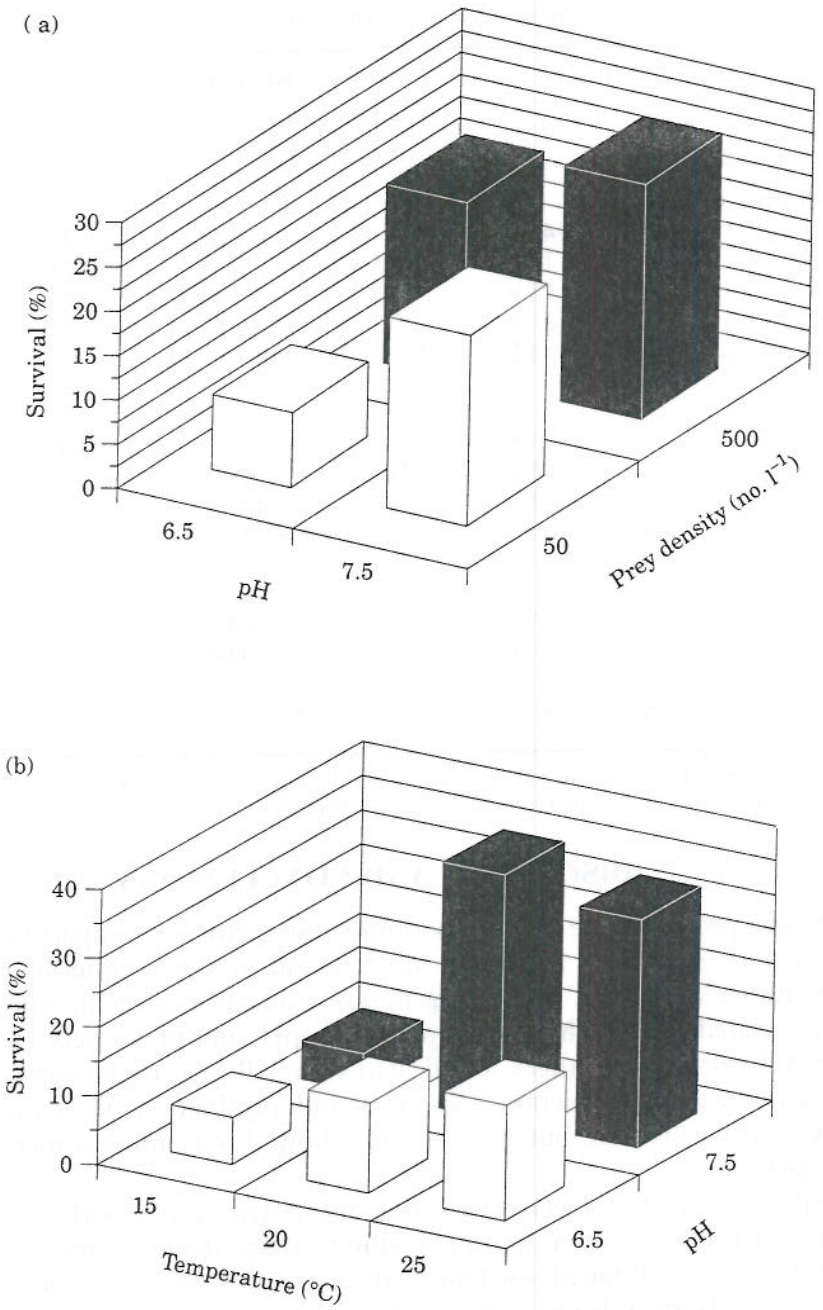

FIG. 3. American shad larvae. Three-factor combination (long-term) experiment, mean survivals at 20 days posthatch for (a) pH × prey level interaction effects, and (b) temperature × pH interaction effects.

Variation in length and weight

Coefficients of variation in standard lengths and weights, which were analysed to determine if variation in larval sizes at the end of the experiment differed among treatments, did not differ significantly (Table IV). Mean within-treatments coefficients of variation were 9·64 and 36·62% for length and dry weight, respectively.

TABLE V. χ^2 summary of time course of observed and expected mortalities in the three-factor combination experiment

Treatment	Days posthatch				Σ
	5–8	9–12	13–16	17–20	
Temperature (°C)					
15	40·3	3	33·2	0·2	76·7
20	13·2	2·1	5	0·01	20·4
25	11·9	0·2	17·9	0·2	30·2
Σ	65·4	5·3	56·1	0·5	127·3
critical $\chi^2_{0\cdot05,6}=12\cdot59$	$\chi^2=127\cdot3$	d.f.=6	($P<0\cdot0001$)		
pH					
6·5	10·6	21·7	6·5	12·4	51·2
7·5	12·1	24·7	7·5	14·2	58·5
Σ	22·7	46·4	14	26·6	109·7
critical $\chi^2_{0\cdot05,3}=7\cdot81$	$\chi^2=109\cdot7$	d.f.=3	($P<0\cdot0001$)		
Prey level					
50 l^{-1}	2	1·7	0·03	0·5	4·2
500 l^{-1}	2·4	2	0·04	0·6	5
Σ	4·4	3·7	0·1	1·1	9·3
critical $\chi^2_{0\cdot05,3}=7\cdot81$	$\chi^2=9\cdot3$	d.f.=3	($P<0\cdot05$)		

χ^2 test of independence for four time periods during the experiment. χ^2 values are in body of table. P, Probability of no difference in mortalities among time periods or factor levels.

DISCUSSION AND CONCLUSIONS

Not unexpectedly, the three environmental factors all impacted clearly on survival and growth of American shad larvae in the long-term, three-factor combination experiment. Increasing trends in survival, growth, and production were associated with the higher temperatures, pH, and prey levels. Temperature and prey levels, in the ranges tested, had especially strong impacts on survival rates, growth and production. Survival and production of larvae improved greatly as pH increased, but growth rates showed only modest increases at the higher pH.

The interaction effects were interesting and potentially important with respect to recruitment success of American shad in Chesapeake tributaries. There was a strong impact of combined low temperature and low prey levels on survival and production. Temperatures approaching 15° C and zooplankton prey levels $<100\ l^{-1}$, conditions that are common during April and early May in Chesapeake tributaries (Houde & Rutherford, 1992; Rutherford & Houde, 1995; Secor & Houde, 1995), are not favourable to American shad larvae. The low temperature–low pH and low pH–low prey level combinations also had severe consequences for survival and production of larval American shad and represent conditions that can occur in Chesapeake tributaries during spawning or hatchery-release seasons.

The 48-h shock experiments demonstrated strong responses in survival probability of American shad larvae to environmental pulses of temperature and

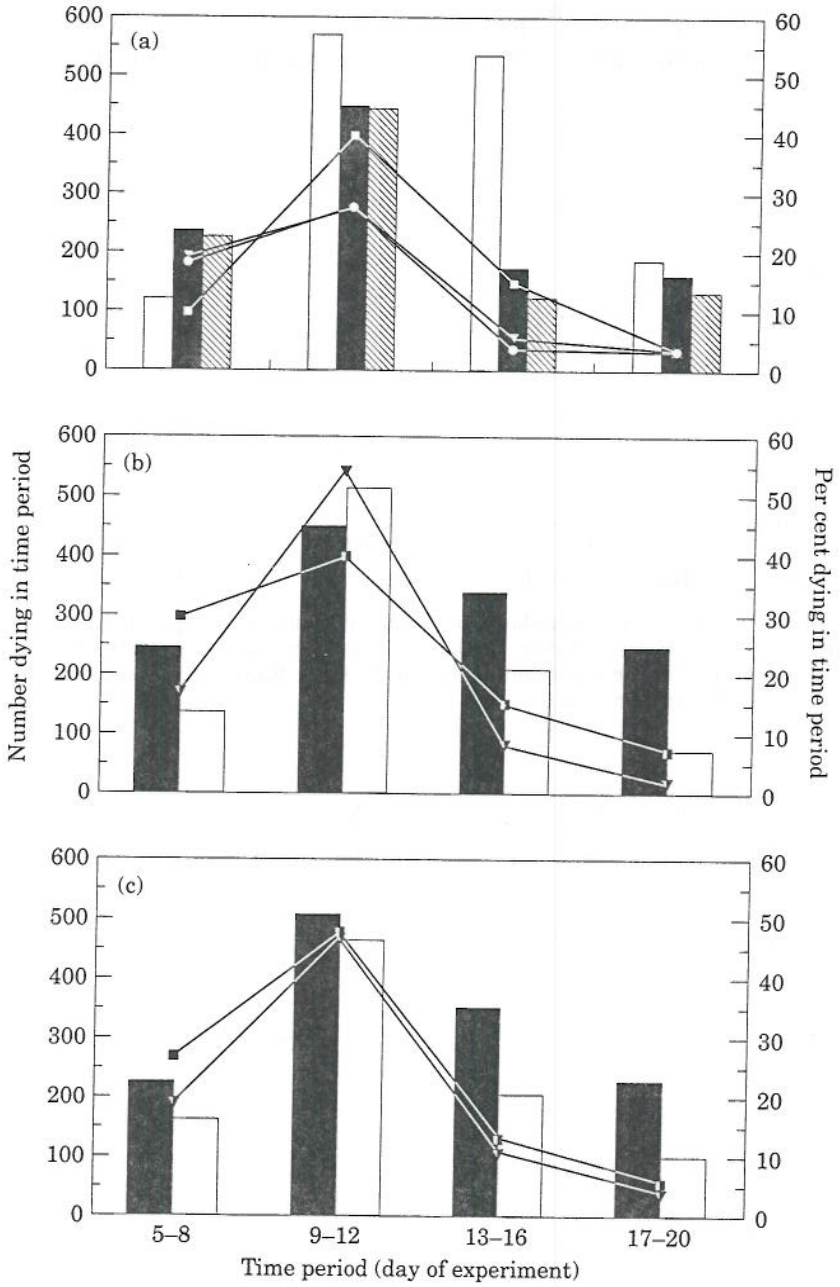

FIG. 4. American shad larvae. Three-factor combination (long-term) experiment, time course of mortalities and bar chart of mean percent mortality for four time periods during the experiment for (a) temperature effects, (–■– □), 15°C; (–▼– ■), 20°C; (–●– ▨), 25°C; (b) pH effects, (–■– ■), pH 6·5, (–▼– □), pH 7·5 and (c) prey level effects, (–▼– ■), 50 prey l^{-1}; (–■– □), 500 prey l^{-1}.

pH. Quick rises in temperature from 20 to 25° C or 20–30° C, or quick depressions of pH from 7·0 to 6·0, were clearly detrimental to feeding-stage larvae, and combined temperature increase and pH depression shocks of those magnitudes often were lethal. It is unlikely that temperature episodes, especially rising temperatures, as dramatic as those tested would occur often in Chesapeake Bay tributaries, except possibly in heated effluent plumes of power plants. But,

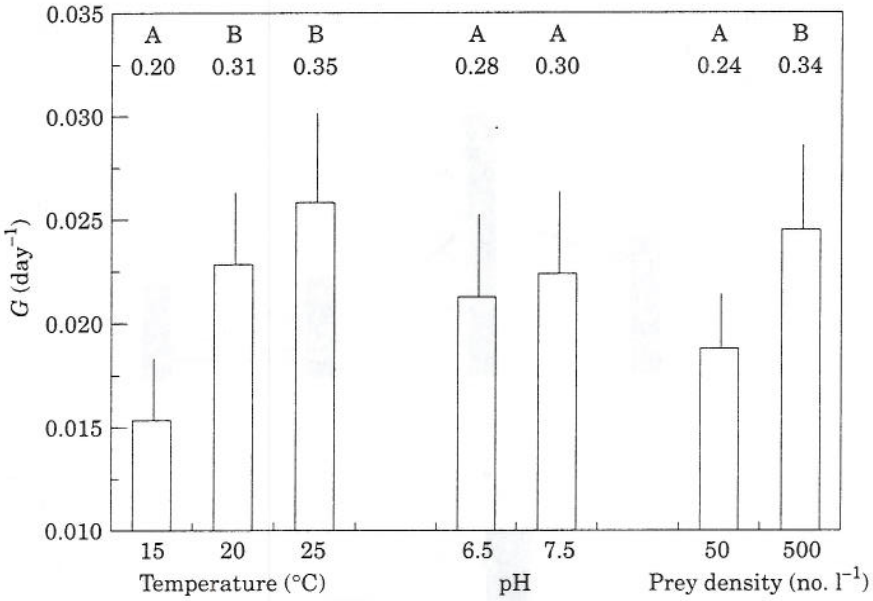

FIG. 5. American shad larvae. Three-factor combination (long-term) experiment, mean length-specific growth rates from 4 to 20 days posthatch. Error bars are +2 S.E. Different letters above bars indicate means that are significantly different ($P<0{\cdot}05$). Rates listed below significance letters are mean growth rates in mm day^{-1}.

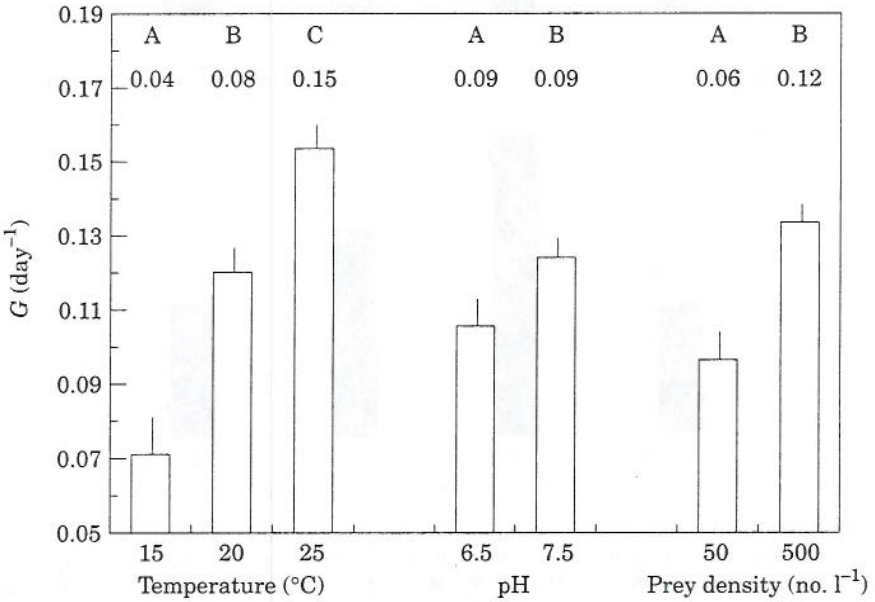

FIG. 6. American shad larvae. Three-factor combination (long-term) experiment. Least-squares mean weight-specific growth rates from 4 to 20 days posthatch. Error bars are +2 S.E. Different letters above bars indicate means that are significantly different ($P<0{\cdot}05$). Rates listed below significance letters are mean growth rates in mg day^{-1}.

pH depressions similar to those imposed may occur, usually in association with rainfall events (Hall *et al.*, 1985), indicating a potential for high and sudden mortalities of American shad larvae stocked or hatched into such conditions.

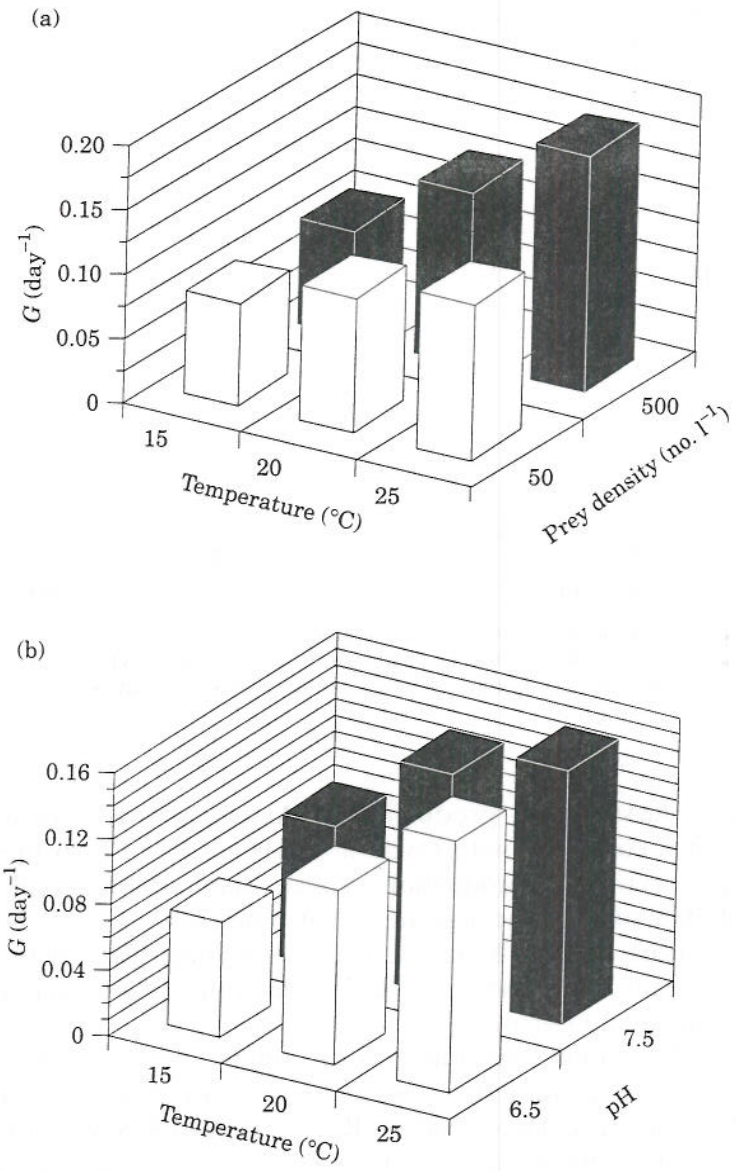

FIG. 7. American shad larvae. Three-factor combination (long-term) experiment, mean weight-specific growth rates from 4 to 20 days posthatch for (a) temperature × prey level interaction effects, and (b) temperature × pH interaction effects.

The results generally supported earlier laboratory studies, which indicated that American shad larvae survival is best at temperatures in the range 15·5–26·1° C and pH>6.7 (Klauda *et al.*, 1991). Survival rates were reasonably good at pH 6·5 when temperatures were ≥20° C, and prey level was 500 l^{-1}, but dropped precipitously at 15° C and 50 prey l^{-1}. The results build upon Johnson & Dropkin's (1995) conclusion that American shad larval growth and survival are sensitive to levels of prey. The shock experiments suggested that episodic increases in temperature were more detrimental to both feeding-stage and yolk-sac larvae than were sudden temperature drops. In contrast, the long-term experiments demonstrated that chronic low temperature (15° C) was not

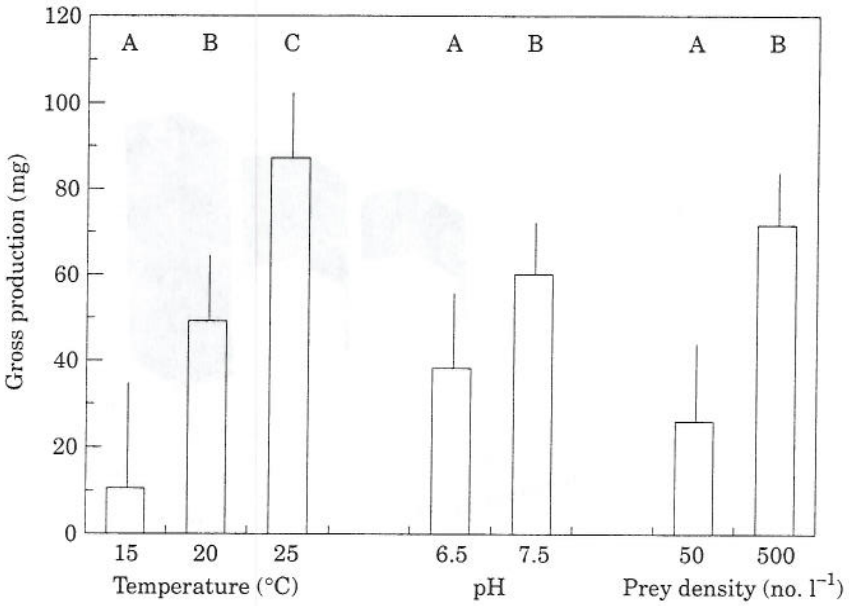

FIG. 8. American shad larvae. Three-factor combination (long-term) experiment. Least-squares mean gross production from 4 to 20 days posthatch. Error bars are +2 S.E. Different letters above bars indicate means that are significantly different ($P<0{\cdot}05$) in analysis of log-transformed gross production.

conducive to survival and production of American shad larvae, while 25° C, especially when combined with favourable pH (7·5) and high prey availability, favours high survival and production. Taken together, these results and those of previous studies support the argument that timing of spawning by adults under natural conditions (Crecco & Savoy, 1987*b*; Limburg, 1996) or timing of release of hatchery-origin larvae can be critical in determining whether American shad larvae will survive and grow.

High river flow conditions and low, or falling, temperatures, combined with low prey levels, are conditions associated with poor survival of American shad larvae in the Connecticut and Hudson Rivers (Crecco & Savoy, 1987*a*; Limburg, 1996). In the long-term experiments, it was clear that larvae fared better at 20–25° C than at 15° C and that survival and production generally were much higher at pH 7·5 than at pH 6·5. Based upon recent research on striped bass early-life dynamics in Chesapeake Bay tributaries (Houde & Rutherford, 1992; Rutherford & Houde, 1995; Secor & Houde, 1995; Kellogg *et al.*, 1996) temperatures in the 20–25° C range are likely to occur from mid-May to early June in Maryland tributaries of Chesapeake Bay, and both pH and prey levels are likely to be higher after mid-May than earlier. Thus, it is probable that hatchery-origin American shad larvae would fare better if released into rivers after mid-May than before that period. In the northernmost portions of the upper Chesapeake Bay, late May and early June release dates almost certainly would be best.

The potential differences in survival and production of young American shad, in response to environmental conditions in the range that they might experience, are not trivial. Based upon the experimental results, several-fold differences in

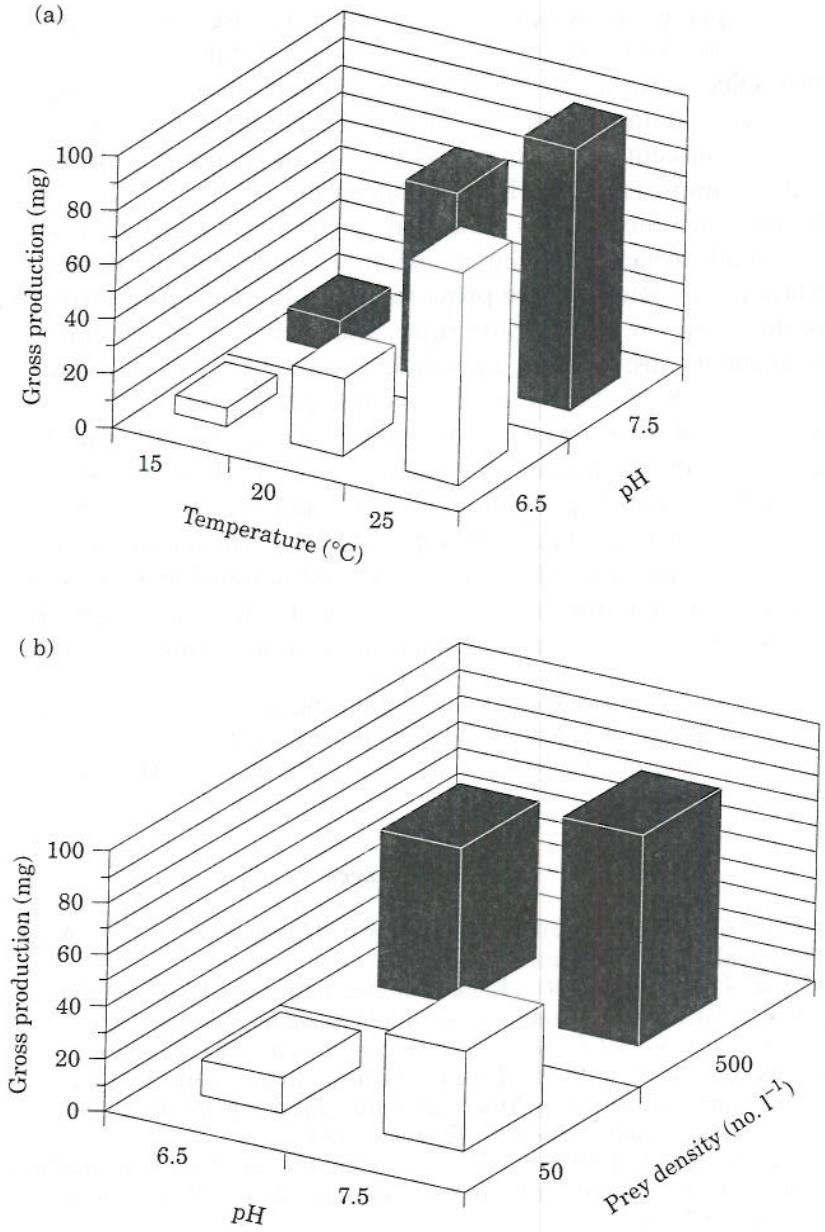

FIG. 9. American shad larvae. Three-factor combination (long-term) experiment, mean gross production from 4 to 20 days posthatch for (a) temperature × pH interaction effects, and (b) pH × prey level interaction effects.

larval survival and production may be expected under environmental conditions that are probable in Chesapeake Bay tributaries during spring months. In restoration efforts, selecting appropriate dates and places to stock American shad larvae clearly is important to ensure reasonable survival and eventual return.

Predation is another potential source of mortality and loss. Johnson & Dropkin (1992) demonstrated that predators can remove a large fraction of stocked American shad larvae. Predation pressure on larvae could increase

during late spring, as water temperatures increase and as predators, both invertebrates and fish, increase their consumption rates. Thus, there may be a tradeoff in selecting dates to stock American shad larvae. Stocking too early can expose larvae to unfavourable environmental conditions; stocking too late may increase their susceptibility to predation, although there is no estimate of the magnitude or importance of the tradeoff effect.

Field tests are needed to evaluate survival and production of stocked American shad larvae under environmental conditions in the tributaries. The present laboratory studies have provided a baseline of information that can help to make decisions on appropriate times and places to release larvae. However, field experiments are needed in which larvae are released as environmental probes to evaluate specifically environmental quality in the tributaries, judged by larval survival. Release times and places then can be compared, based upon recapture rates and estimates of relative survival. The age (size) of larvae at release is yet another important factor that requires further evaluation under field conditions in Chesapeake Bay tributaries. Ultimately, a combination of laboratory experiments and field tests is needed to develop a knowledge of larval tolerance levels to environmental factors and to develop restoration protocols that will optimize survival and production of young American shad.

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