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A Laboratory Study of Nongenetic Embryonic Adaptation to Salinity and Its Subsequent Effects Upon Larval Development of the Grass Shrimp *Palaemonetes pugio* Holthius

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A LABORATORY STUDY OF NONGENETIC EMBRYONIC ADAPTATION TO SALINITY AND ITS SUBSEQUENT EFFECTS UPON LARVAL DEVELOPMENT OF THE GRASS SHRIMP *Palaemonetes pugio* Holthuis

by

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ABSTRACT

A LABORATORY STUDY OF NONGENETIC EMBRYONIC ADAPTATION TO SALINITY AND ITS SUBSEQUENT EFFECTS UPON LARVAL DEVELOPMENT OF THE GRASS SHRIMP *Palaemonetes pugio* Holthuis

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Director: Dr. John R. McConaugha

Fertilized embryos of the grass shrimp *Palamonetes pugio* Holthuis (Decapoda; Caridea) were exposed to two levels of salinity [5 ppt (exposed) and 20 ppt (control)] during incubation to determine the extent to which embryonic adaptation influences subsequent larval development. Larval response to embryonic exposure was measured at three salinities (5 ppt, 10 ppt, and 20 ppt) as the fraction of larvae surviving to metamorphosis and the duration (in days) of larval development. The survival rate of larvae hatched from control, or non-adapted, embryos was significantly influenced by rearing salinity. The differences in survival of pre-adapted larvae (exposed group), however, were not significant at any of the three rearing salinities. In the control group, mean survival rates at 5 ppt, 10 ppt, and 20 ppt were 84.2%, 95.8%, and 100%, respectively. In the exposed group, mean survival at the same rearing salinities were 93.6%, 98.2%, and

96.6%, respectively. Between-group comparisons (i.e. exposed versus control) show that at 5 ppt larvae hatched from pre-adapted embryos exhibited significantly enhanced survival when compared to larvae hatched from control embryos. Conversely, at 20 ppt survival of pre-adapted larvae was significantly diminished. Larval duration was not significantly influenced by salinity in either experimental group. The median time to metamorphosis was 14.80 days in the control group and 14.67 days in the exposed group.

DEDICATION

This thesis is dedicated to my mother, whose faith in me has never waivered, and to the memory of my father, whose words of encouragement and inspiration will never be forgotten. Their devotion, patience, and moral support have made it all possible.

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A word of thanks must certainly go to the faculty and staff of the Department of Oceanography who have shared their talents and knowledge and have made this graduate education a very meaningful component of my life. Special thanks go to Dr. Ronald E. Johnson, Graduate Program Director, for his consistent and sincere demonstration of concern for the student's well-being.

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Chapter 1

INTRODUCTION

Laboratory investigations of the environmental physiology and ecology of decapod crustacean larvae have traditionally dealt with larval responses to environmental parameters as measured by the fraction of an initial population of larvae surviving to metamorphosis. Perhaps even more important, though, and certainly of more ecological significance, are the sub-lethal responses to these parameters. Some of these responses may be measured as duration of larval development, size at metamorphosis, number of molts required to reach metamorphosis, intermolt duration, morphological alterations, and behavior. Localized daily (tidal) fluctuation in salinity is a conspicuous characteristic of an estuary and, depending upon the range of this fluctuation, salinity most certainly influences larval development in nature. The ecological success of an estuarine organism, such as the grass shrimp Palaemonetes pugio Holthuis, is closely linked with the degree to which its larval forms are able to tolerate fluctuations in salinity. It has been suggested that the ability of an individual larva to tolerate a salinity fluctuation may have indeed been influenced by that

individual's environmental or adaptive history (Kinne, 1964).

Nongenetic adaptation, or acclimation, involves changes in an individual's response mechanism due to direct environmental influence, and it is assumed that this adaptation is not passed on to the next generation. Genetic adaptation is closely related but involves changes in the genotype. Adaptive capacity and associated mechanisms are, in essence, products of natural selection. In that regard, nongenetic and genetic adaptation exhibit differences which are sometimes subtle and difficult to distinguish. It has been suggested that the capacity for nongenetic adaptation is greatest during early ontogenetic development and that "early" adaptation may even be partially irreversible for the remainder of life of that individual (Kinne, 1962; 1964; and Rosenberg and Costlow, 1979).

The grass shrimp, Palaemonetes pugio Holthuis, is a conspicuous inhabitant of coastal estuaries ranging from Massachusetts to Texas (Williams, 1965) and plays a significant role in the detrital food web of some estuarine communities (Welsh, 1975). The Caridea, in general, is the dominant natant decapod group found in temperate regions (Allen, 1966). Investigations of the larval development and ecology of Palaemonetes spp. are certainly too numerous to review comprehensively in this paper. However, several are particularly relevant to the

present study. In laboratory studies, Floyd (1977) and Sandifer (1973) have shown significant effects of salinity upon the larval development of P. pugio and P. vulgaris, respectively. In these studies, at 25°C, rates of larval survival were highest at salinities between 20 ppt and 30 ppt. Survival to metamorphosis, when larvae were reared at 5 ppt, was significantly reduced. Both studies showed significant independent and interactive effects of temperature and salinity upon survival. Broad and Hubschman (1962) also found poor larval survival of P. pugio, P. vulgaris, and P. intermedius at salinities below 10 ppt. Studies of embryonic adaptation to salinity in crustaceans are very limited and the author is not aware of any such studies involving palaemonid shrimp. The subjects of nongenetic adaptation to temperature and salinity and irreversible nongenetic adaptation are treated in detail by Kinne (1962, 1964). Several authors have provided comprehensive reviews of research carried out in the general area of adaptations to environmental variables (Kinne, 1963, 1970, 1971; Carriker, 1967; Vernberg, 1972, 1975; Lockwood, 1976; Newell, 1976; and Sastry and Vargo, 1977).

Possible evidence for nongenetic adaptation has been provided for the desert pupfish, Cyprinodon macularis, and the brackish-water amphipod, Gammarus duebeni (Kinne, 1953, 1958, 1962); the Caridean shrimp, Crangon crangon (Broekema, 1941); and the euryhaline barnacle, Balanus amphitrite

amphitrite (Crisp and Costlow, 1963). Rosenberg and Costlow (1979) exposed embryos of the branchyuran crab, Rhithropanopeus harrisii Gould to certain temperature/salinity combinations in order to determine the effect on larval development at the same conditions. In that study, acclimation of the eggs at 30 ppt had a significant effect upon subsequent larval survival at 5 ppt.

In the present study, fertilized embryos of the grass shrimp, Palaemonetes pugio Holthuis (Decapoda, Caridea) were exposed to a level of salinity known to be stressful to larvae of this species under laboratory-rearing conditions in order to determine the extent to which nongenetic embryonic adaptation affects subsequent larval development. P. pugio is quite well suited for these experiments, as adults are easily collected locally due to the species' successful exploitation of shallow water habitats in the lower Chesapeake Bay tributaries. These shrimp are easily maintained in the laboratory and the reproductive biology, especially with regard to controlled matings, is fairly well-established (Little, 1968; Boston, 1978; and Buikema, 1980). The larvae are well-suited for laboratory rearing and their temperature/salinity tolerance limits and optima have been documented by Floyd (1977). Finally, Palaemonetes spp.--adults and larvae--have been widely used in bioassay work; therefore, any information contributing towards a better understanding of Palaemonetes' responses to

stressful environmental conditions becomes valuable in the planning or designing of future research.

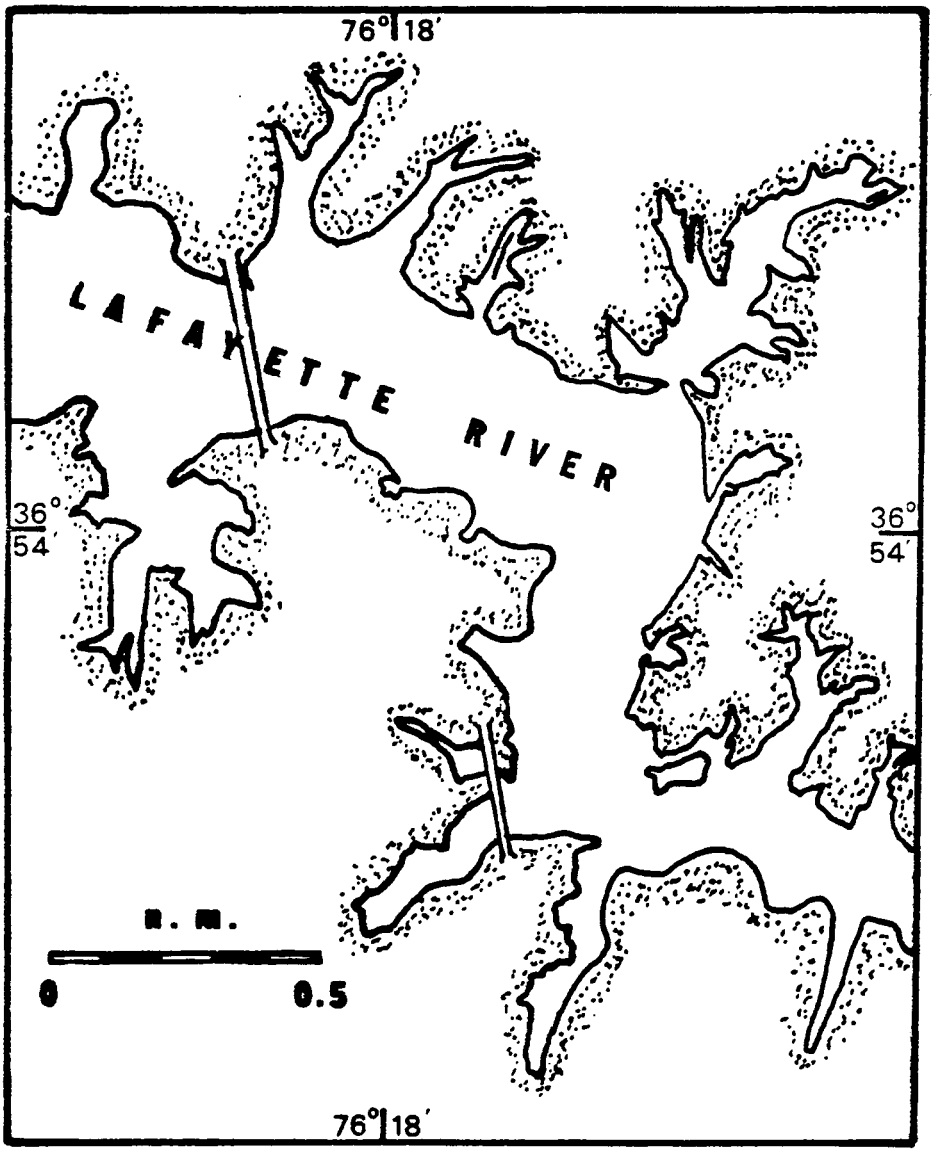
Chapter 2

MATERIALS AND METHODS

Collection and Maintenance of Adults

Adult Palaemonetes pugio were collected with push nets from the Lafayette River, Norfolk, Virginia (Fig. 1) in the fall of 1980. Collections were made at low tide and specimens were returned to the laboratory in buckets containing water from the collection site (temperatures ranging from 20°C to 25°C). The temperature of the water holding newly-collected animals was brought to 25°C (±1 C) over a period of 24-36 hours (aeration provided). The adults were then sexed according to pleopod characteristics (Meehan, 1936) and arbitrarily separated into two groups: 20-30 animals in each (≈1:1 sex ratio). Each group was placed in separate ten-gallon aquaria with undergravel, biological filters, housed in a Percival Model I-35L Incubator. Both aquaria were maintained at 25°C under a 15 hour light: 9 hour dark photoperiod to aid in inducement of breeding activity. The first group ("control") was maintained at a salinity of 20 ppt and the second ("exposed") at 5 ppt. A stepwide acclimation (5 ppt per hour) to these salinities was sometimes carried out since salinities at the collection site typically ranged from

Fig. 1. Lafayette River (Norfolk, Virginia). Location of collection site of adult Palaemonetes pugio.


76° 18′
LAFAYETTE RIVER
36° 54′
36° 54′
N. M.
0
0.5
76° 18′

14 ppt to 18 ppt. All seawater used in these experiments was collected offshore, filtered through Gellman glass fiber filters, and diluted with de-ionized water. Food was offered daily in the form of frozen fish, shrimp, detritus, and live Artemia salina nauplii and adults. Ovarian development in females was observed daily in order to monitor progress towards mating and subsequent egg deposition. The period of time required for complete ovarian development was, naturally, dependent upon their stage of development at the time of collection. This period ranged from 5 to 25 days. In preliminary investigations, it was found that handling females shortly after egg deposition typically resulted in her dropping her eggs. Therefore, females with new broods were allowed to remain in maintenance aquaria throughout most of the period of embryonic development. Their broods were observed daily and when embryos showed signs of advanced development (i.e. dark coloration and formation of eye spots), the female was then isolated in a four liter plastic aquarium with moderate aeration. When hatching appeared imminent, she was placed in a hatching chamber (submerged within the isolation aquarium) specifically designed so that freshly hatched zoeae would drop through the protective mesh, thus escaping ingestion by the female parent (Fig. 2). The brooding period ranged from 11 to 15 days.

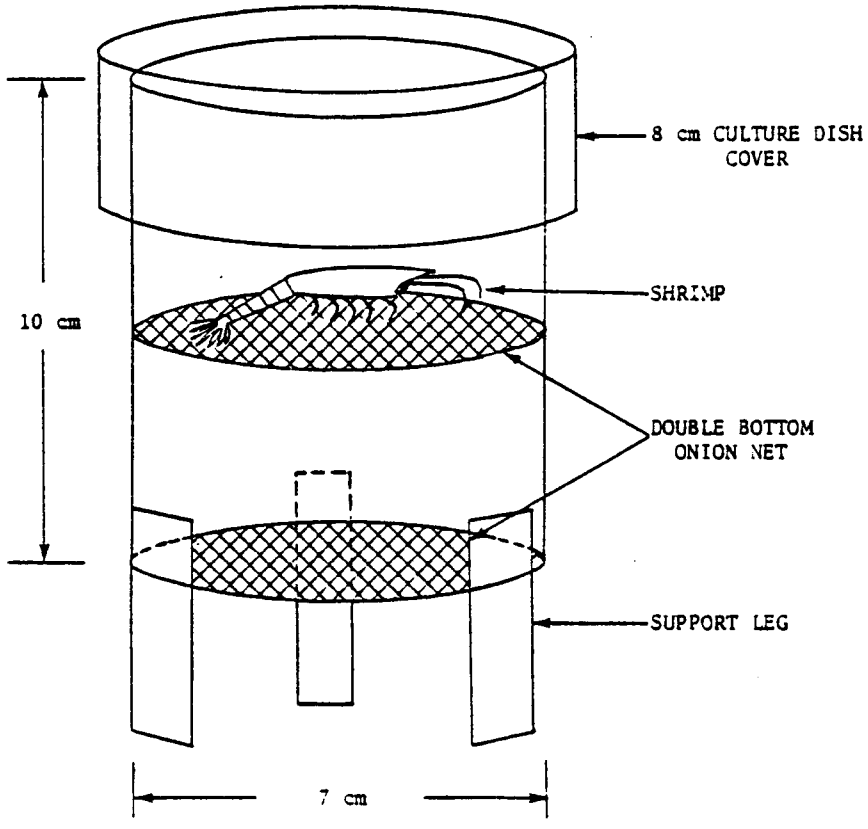


Fig. 2. Schematic diagram of female isolation and hatching chamber.

Larval Rearing

Embryos usually hatched overnight. On the morning following hatching, the female was removed and larvae were transferred by pipette to one liter of filtered seawater in 20 cm-diameter glass culture dishes at a density of approximately 36 larvae per dish. Total hatch size was determined at this time. Larvae which remained motionless after gentle prodding were not used in these experiments. Acclimation to test conditions took place at this time as well as the recording of the total number of larvae per hatch (not including those larvae unresponsive to mild stimuli). Larvae were transferred daily (via large-bore pipette) to clean culture dishes with new seawater and fed live, freshly-hatched Artemia salina (San Francisco Bay Brand) nauplii in excess. Larvae were maintained in total darkness to ensure an even spatial distribution of Artemia nauplii (no phototactic response) at 25°C.

Daily records were kept to monitor rate of development and mortality. Larvae were reared to metamorphosis, thus allowing the determination of percent survival to metamorphosis and duration of larval development. Percent survival was calculated as the fraction of the initial population surviving the molt to post-larva. The duration of larval development was expressed as the amount of time, in days, that zoeae endure prior to metamorphosis. In rare instances where stranding mortalities occurred, these larvae were not included in the total population. For the

purpose of statistical analysis, survival percentages were angularly transformed: response, Y = arcsin ($\sqrt{\text{percent survival}}$). This transformation acts to stabilize error variance by expanding the tails and compressing the middle of the binomial distribution (Sokal and Rohlf, 1969). Data for transformed percent survival and development time were treated with a one-way single classification analysis of variance (ANOVA) for detection of differences in larval survival and larval duration between and within groups (Sokal and Rohlf, 1969). Paired comparisons of transformed survival data for the three rearing salinities were made with the F-test in the control group and with tests of least significant difference (unequal n) in the exposed group.

Experimental Design

It was the basic purpose of this study to determine the effects of exposing embryos to a salinity known to be stressful to larvae of Palaemonetes pugio and subsequently rearing these 'pre-adapted' larvae under sub-optimal and optimal conditions. Floyd (1977), in this investigation of the temperature-salinity tolerance of P. pugio larvae, demonstrated 85.7% survival to metamorphosis at 25°C and 20 ppt. Larval mortality was very high at 5 ppt (5.7%), supporting the present use of this level as a sub-optimal or 'stressful' condition.

The 'exposed' group consists of embryos acclimated to 5 ppt throughout development (from fertilization to hatching). After hatching, larvae from each individual female were separated into three salinity test groups (5 ppt, 10 ppt, and 20 ppt) and reared to metamorphosis at these salinities. Larvae were acclimated to test salinities of 10 ppt and 20 ppt by exposing them to stepwise increases in salinity at a rate of 5 ppt per hour. Larvae acclimated in this manner showed no visible signs of distress. In situations where the hatch size fell below 90 individuals (30 larvae per rearing condition), the hatch was divided into three equal portions for subsequent exposure to each rearing salinity. In the control group, embryonic development was maintained at 20 ppt (25^{o}C) and larvae were treated precisely as in the experimental group. Four separate females and their broods were treated as replicates for each test condition.

Chapter 3

RESULTS

Larval Survival to Metamorphosis

The survival rate of larvae hatched from control, or non-adapted, embryos was significantly influenced by rearing salinity ($P<<0.001$). The differences in survival of pre-adapted larvae (i.e. larvae hatched from embryos exposed to 5 ppt throughout incubation), however, were not significant ($P = 0.05$) at any of the three separate rearing salinities. Results of the ANOVA performed upon arcsine transformed percent survival data for both experiment groups are shown in Appendix 1. Tests of significance (F-tests and Least Significant Difference) between individual rearing salinities confirm significant differences in larval survival within the control group and no significant differences in larval survival within the pre-adapted group (Appendix 2).

Mean survival to metamorphosis of larvae from the control group reared at 5 ppt, 10 ppt, and 20 ppt was 84.2%, 95.8%, and 100.0%, respectively. Mean survival to metamorphosis of larvae from the pre-adapted group reared at 5 ppt, 10 ppt, and 20 ppt was 93.6%, 98.2%, and 96.6%, respectively. Descriptive statistics (mean, variance,

standard deviation, and range) for each experimental group reared at each salinity are shown in Table 1 and Figure 3.

Between-group comparisons were performed for each rearing salinity (Appendix 3 and Fig. 4). At 5 ppt, larvae hatched from pre-adapted embryos exhibited significantly ($P < 0.05$) enhanced survival when compared to larvae hatched from control embryos. Conversely, at 20 ppt, survival of pre-adapted larvae was significantly diminished. At the intermediate rearing salinity, 10 ppt, no significant difference in larval survival to metamorphosis was observed.

Duration of Larval Development

The time (in days) required for larval development, from hatching to metamorphosis, was not significantly influenced by salinity in either experimental group (Appendix 4). Mean larval duration ranged from 14.50 to 16.28 days in control group and from 14.32 to 16.36 days in pre-adapted group (Table 2). Median time to metamorphosis between both groups differed by only 0.13 day (Table 2 and Fig. 5). Figure 6 (p. 20) depicts relative proportions of larvae, from both groups, attaining metamorphosis on individual days, at each rearing salinity.

Embryonic Development

The duration of embryonic development (at 25°C) ranged from 11 to 15 days ($\bar{x}$ =13.25 days) when embryos were incubated at 20 ppt and from 11 to 14 days ($\bar{x}$ =12.25 days) when incubated at 5 ppt. The number of larvae hatching

Table 1. Means (untransformed) and standard deviations of percent larval survival of two groups of Palaemonetes pugio with different adaptive histories and reared at three salinity levels.

		Rearing Salinity		
Group	Replicate	5 ‰	10 ‰	20 ‰
CONTROL:	1	82.05*	94.12	100.00
	2	88.89	94.44	100.00
	3	80.56	87.22	100.00
	4	85.29	97.22	100.00
	$\bar{x}$	84.20	95.75	100.00
	s	3.70	1.70	0.00
EXPOSED:	1	85.71	--	94.74
	2	94.44	100.00	94.12
	3	97.06	97.37	100.00
	4	97.37	97.37	97.37
	$\bar{x}$	93.64	98.25	96.56
	s	5.45	1.52	2.69

*Mean percent survival of metamorphosis (untransformed).

Fig. 3. Mean (horizontal bar) ± one standard deviation (box) and range (vertical bar) of larval survival (untransformed) to metamorphosis for Palaemonetes pugio reared at three salinity levels. Control group represents larvae hatched from embryos adapted to 20 ppt. Exposed group represents larvae hatched from embryos adapted to 5 ppt.

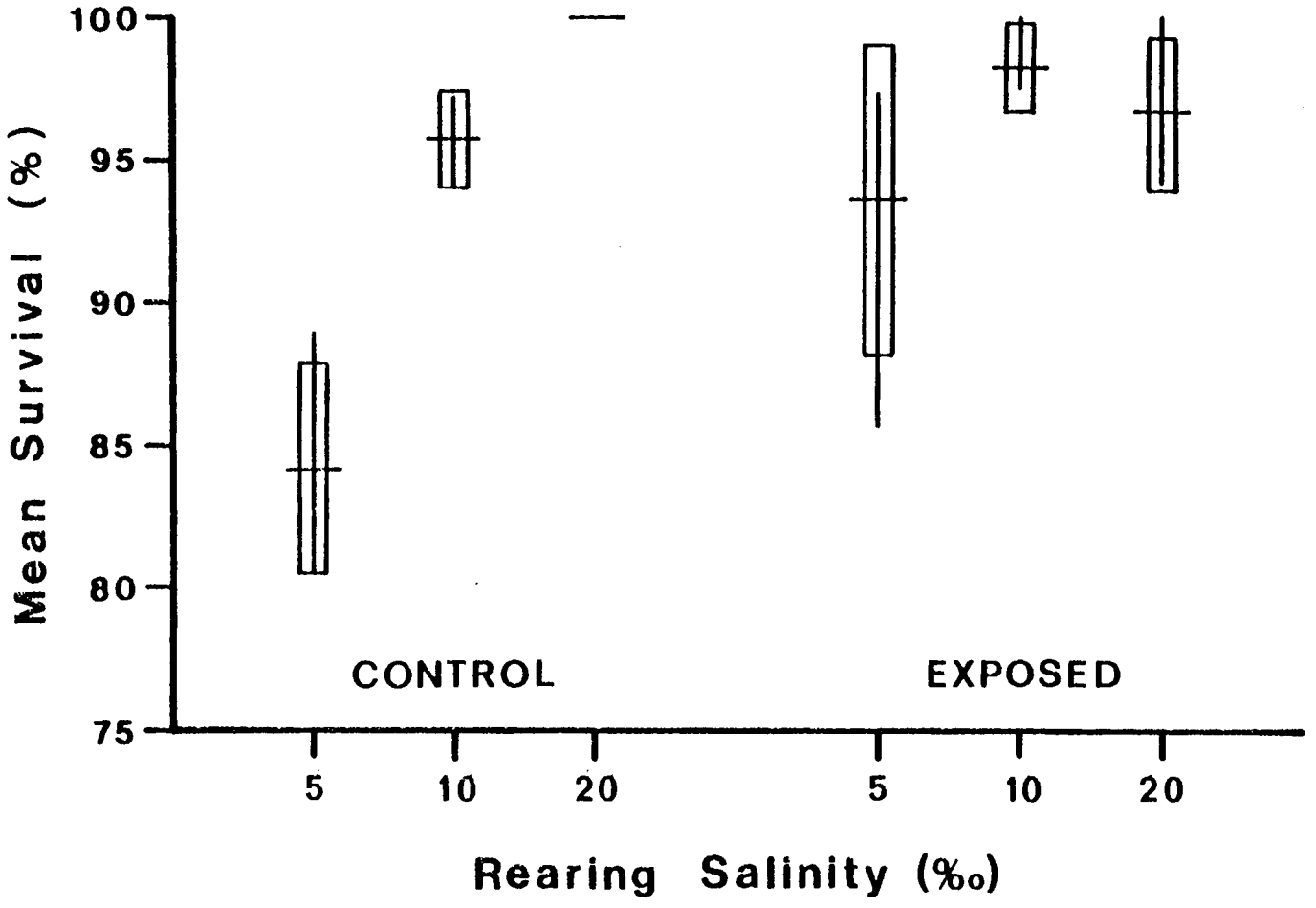

100
95
90
85
80
75
Mean Survival (%)
CONTROL
EXPOSED
5
10
20
5
10
20
Rearing Salinity (‰)

Fig. 4. Comparison of mean percent survival between control and exposed groups at each larval rearing salinity. Numerals at top of bars indicate total number of larvae surviving to metamorphosis.

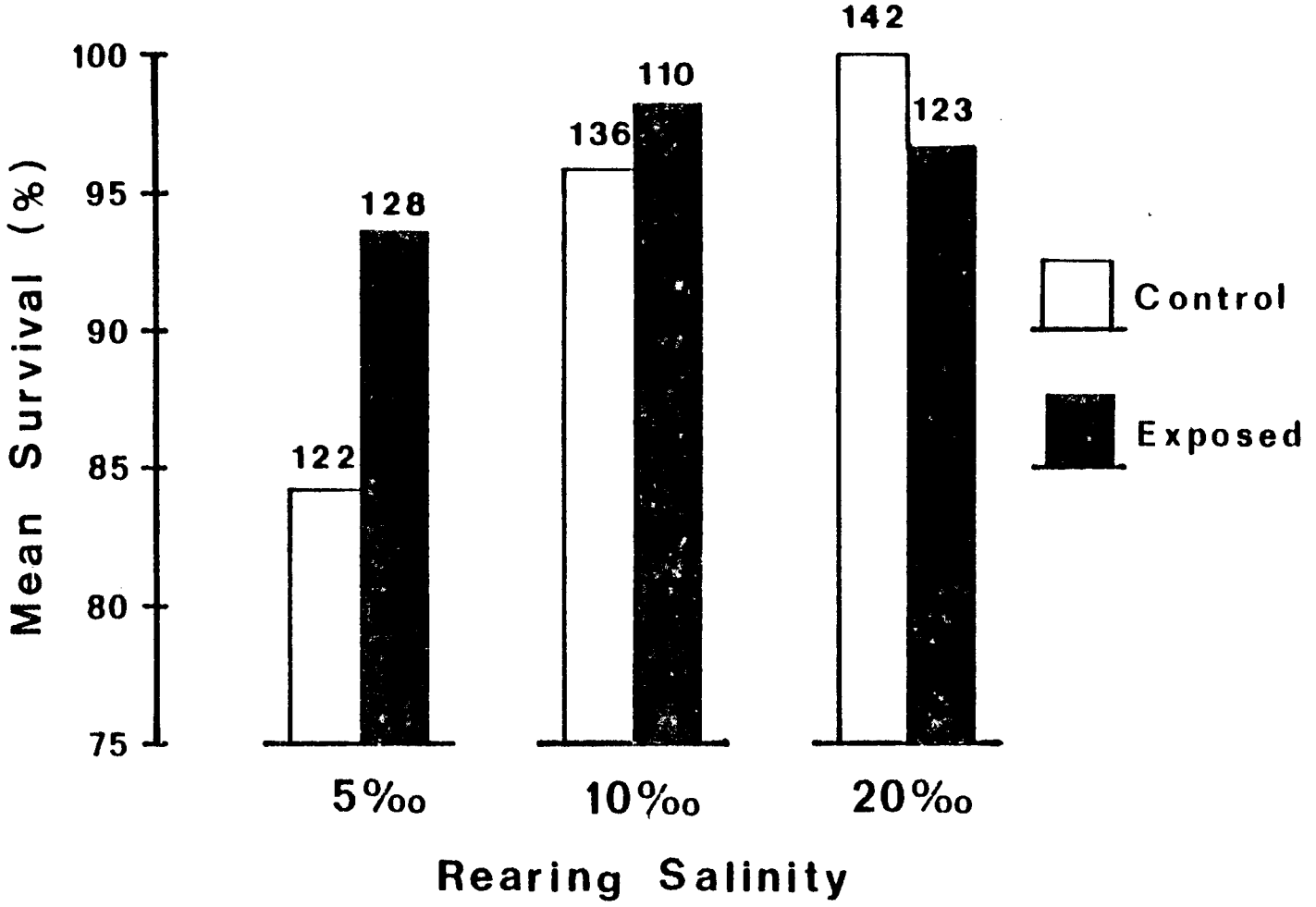

100
95
90
85
80
75
Mean Survival (%)
122
128
136
110
142
123
Control
Exposed
5‰
10‰
20‰
Rearing Salinity

Table 2. Means and standard deviations for duration of larval development of two groups of Palaemonetes pugio with different adaptive histories and reared at three salinity levels.

		Rearing Salinity			
	Replicate	5 ‰	10 ‰	20 ‰	
CONTROL:	1	15.19*	14.72	15.23	$\bar{x}$: 15.27
	2	14.97	14.50	15.00	s: 0.53
	3	16.28	15.83	15.86	median: 14.80
	4	15.62	14.83	15.25	
	$\bar{x}$	15.52	14.97	15.34	
	s	0.58	0.59	0.37	
EXPOSED:	1	14.67	--	14.78	$\bar{x}$: 15.26
	2	14.32	14.61	14.47	s: 0.77
	3	16.36	15.84	16.36	median: 14.67
	4	16.03	15.08	15.32	
	$\bar{x}$	15.34	15.18	15.23	
	s	1.00	0.62	0.83	

*Mean number of days to metamorphosis.

Fig. 5. Cumulative percentage of larvae of Palaemonetes pugio attaining metamorphosis on each day. Survival curves for control and exposed groups represent pooled data from all three rearing salinities within each group.

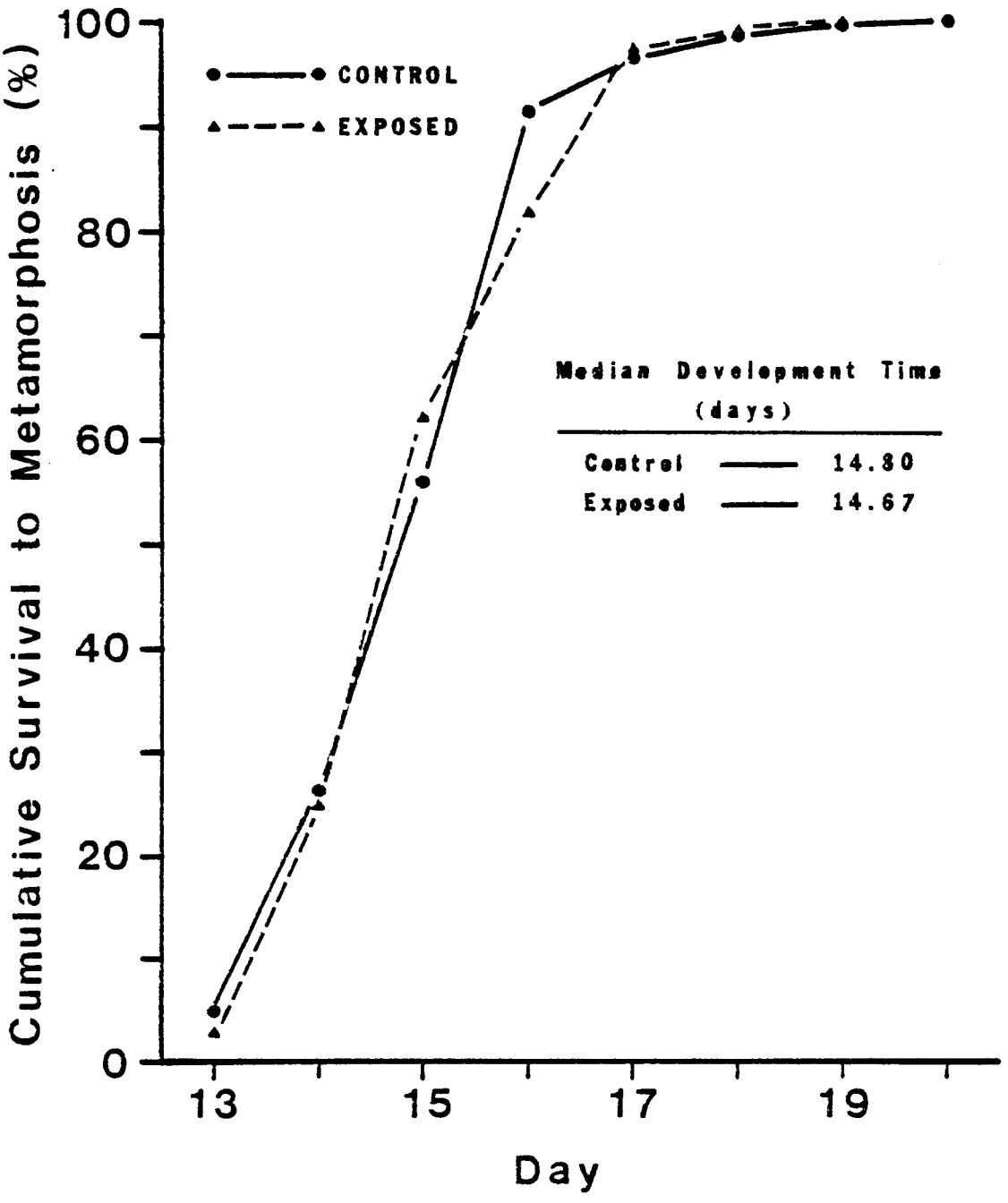
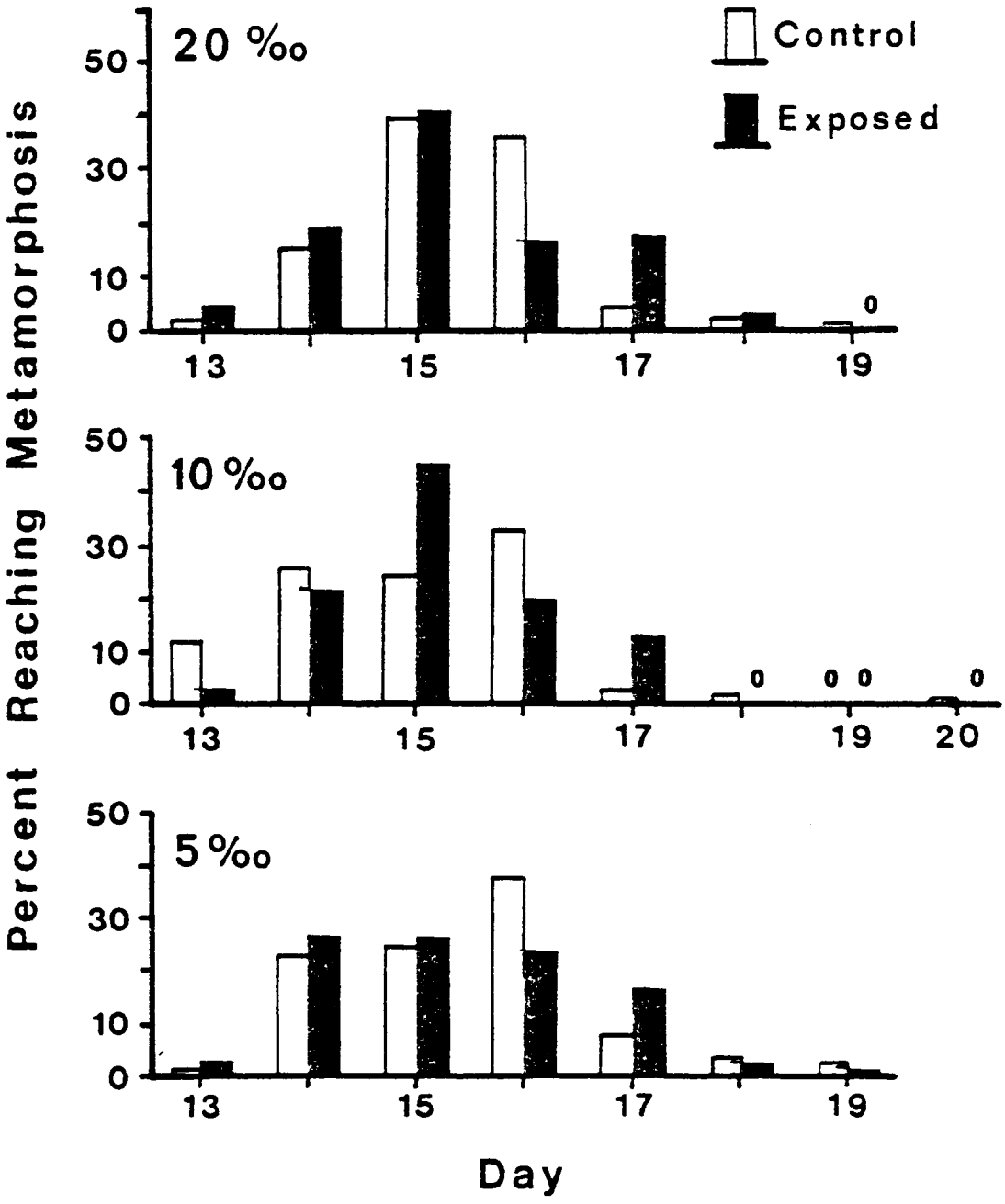

Cumulative Survival to Metamorphosis (%)
100
80
60
40
20
0
13
15
17
19
Day
CONTROL
EXPOSED
Median Development Time
(days)
Control 14.80
Exposed 14.67

Fig. 6. Percentage of larvae of Palaemonetes pugio from control and exposed groups attaining metamorphsis each day at each of three rearing salinities.


20 ‰
Control
Exposed
50
30
10
0
13
15
17
19
0
10 ‰
50
30
10
0
13
15
17
19
20
0
0 0
0
5 ‰
50
30
10
0
13
15
17
19
Percent Reaching Metamorphosis
Day

successfully from each brood ranged from 125 to 230 in the control group and from 50 to 140 in the pre-adapted group (Table 3). The difference in mean hatch size between both groups was not significant ($P \leq 0.05$).

Table 3. Mating histories of eight female *Palaemonetes pugio* adults used in laboratory rearing experiments.

Group	Female	Date Eggs Deposited	Date Hatched	Duration of Embryonic Development	Hatch Size
CONTROL:	1	13 VIII 80	28 VIII 80	15 days	150
	2	15 VIII 80	29 VIII 80	14 days	125
	3	20 VIII 80	2 IX 80	11 days	160
	4	3 IX 80	14 IX 80	13 days	230
				$\bar{x}$: 13.25	166
EXPOSED:	1	14 VIII 80	27 VIII 80	13 days	50
	2	19 VIII 80	2 IX 80	14 days	126
	3	4 IX 80	15 IX 80	11 days	123
	4	4 IX 80	15 IX 80	11 days	140
				$\bar{x}$: 12.25	110

Chapter 4

DISCUSSION

Previous laboratory investigations of the salinity tolerance of larvae of Palaemonetes pugio have indicated that optimal survival occurs at 20 ppt-25 ppt over a temperature range of 20-30°C (Floyd, 1977). Of particular importance in the present study was the selection of a larval rearing salinity that was certainly sub-optimal. Otherwise, the presence of an adaptive response would have been easily masked. The decision to use 5 ppt as the stressful condition was based upon the results of prior studies. Floyd (1977) reported mean larval survival to metamorphsis at 5 ppt (25°C) to be 5.71%. Other investigations have also shown greatly reduced larval survival (<10% survival to post-larva) at 5 ppt for various species of Palaemonetes inhabiting the estuarine environment (Broad and Hubschman, 1961; and Sandifer, 1973). The present study indicates that, indeed, larval survival to metamorphosis was poorest at 5 ppt. However, the value of mean percent survival (control group) was considerably higher in this study (84.2%). This disparity is certainly significant (especially since adults were collected from the same locations) and brings to light the importance of

two basic differences in rearing conditions between the two studies. First, in the present study, larvae were reared en masse. Floyd (1977) reared larvae individually, one per compartment, in a volume of 25 ml. Although the volume of water available per larvae was only slightly greater in the current study (28 ml), the conditions probably were behaviorally more 'natural.' Second, larvae in the present study were reared in natural seawater. Floyd (1977) prepared synthetic seawater for rearing at various salinities. There is no reason to suspect, however, that artificial media are substantially inferior in laboratory rearing of larval decapods (Sulkin and Minasian, 1973).

Another interesting phenomenon surfaces when the influences of rearing salinity upon larval survival of both groups are compared. Larvae from the control group exhibited significantly different survival rates at all three levels of salinity, with greatest survival at the highest salinity. These results, on a relative basis, are consistent with those of Floyd (1977). A salinity effect is operating distinctly in this case. However, in the group adapted to 5 ppt, this distinction disappears. No significant difference in survival rates exists at any salinity level, the overall effect being enhanced survival of larvae pre-adapted to low salinity (regardless of the level of salinity at which larval development occurred). In nature, such a physiological response may allow larvae

to withstand unusually low levels of salinity, especially in cases in which stressful conditions exist throughout embryonic development and persisting well beyond hatching. This could, in turn, improve recruitment to the parental population as well as recruitment to distant populations farther upstream. Evidences, such as presented in this study, assist in the understanding of the process by which some decapods (such as Palaemonetes spp.) successfully exploit freshwater habitats when all other evidence indicates that larval survival is negligible, at best, at low salinity levels. Broekema (1941) suggested that the salinity in which embryonic development and hatching takes place may later influence the salinity optima of Crangon crangon.

In the estuarine environment, unpredictable, short-term phenomena may have a profound effect on certain organisms (Wells, 1961). Of particular relevance to this study would be the situation in which unusually heavy rainfall markedly reduced shallow-water estuarine salinity. Under these circumstances it is possible that the embryos of an ovigerous female would become acclimated to the sub-optimal salinity. If the low salinities persisted at the time of hatching, the larvae may be better suited for survival at these conditions. Conversely, if conditions have returned to 'normal' by the onset of hatching, and if this nongenetic embryonic adaptation is, at least, partially irreversible, it is feasible that the newly hatched

larvae would encounter difficulty tolerating the normal conditions. Again, it is important to recognize that pre-hatch exposure to sub-optimal conditions may alter or modify larval developmental optima.

The potential effects of salinity stress in combination with sub-optimal levels of other parameters, such as temperature, toxicant concentration, and food availability, could become quite significant under natural conditions. Larval 'populations' developing under stressful conditions (for example, exposed to unusually high concentrations of toxic substances) may be better suited to these conditions when pre-adapted to salinity levels which allow for enhanced survival at that toxicant concentration. Floyd (1977) has shown that, for larval P. pugio, increased survival at critically high temperatures (32.5°C) occurred in the lower salinity range (10 to 20 ppt). These results indicate that embryonic adaptation at low levels of salinity may allow an increase in the upper thermal limits. The study of synergistic effects of environmental variables upon decapod larval development is quite important from the standpoint that our understanding of temperature-salinity optima is typically limited to cases in which the interaction of factors is not included. The response to a specific set of conditions may certainly be modified by a shift in levels of other parameters.

Larvae from the exposed group exhibited enhanced survival when reared at 5 ppt ($P = 0.05$) and 10 ppt

(not significant). Correspondingly, when reared at 20 ppt, survival was significantly diminished ($P = 0.05$). Apparently, the effects of low-salinity embryonic adaptation persists to some degree at even the highest ('optimal') rearing salinity. Mean survival in the exposed group was highest at 10 ppt (Fig. 4, p. 17). Although survival (98.2%) was not significantly higher at this salinity, these results indicate an intermediate effect--perhaps a point at which the effects of low-salinity embryonic adaptations are balanced by the effects of developmental optima.

It is not surprising that mean larval duration differed only slightly under the various exposure regimes. Mean and median time to metamorphosis differed in the two exposure groups by less than one day (Table 2, p. 18). Mean larval duration at 25°C in this study (combined groups) was 15.26 days. At the same temperature, Floyd (1977) shows a mean development time of 19.32 days. This is an appreciable difference in the sense that more time spent as planktonic larvae, on one hand increases the chances of encounter by a predator or of physical disturbance and, on the other hand allows for a greater geographic dispersal, inter-populational mixing, and possibly, colonization of new environments. Several studies have indicated that the duration of larval development is more closely tied to levels of temperature and that basic metabolic processes are temperature-dependent (within a tolerable range);

larval duration decreasing with increasing temperature (Knowlton, 1965; Sandifer, 1973; Christiansen and Costlow, 1975; and Floyd, 1977).

As in the case of larval duration, it appears that the duration of embryonic development was not influenced by exposure salinity (Table 3, p. 22). Embryos in the control and the exposed groups developed for 13.25 days and 12.25 days, respectively, before hatching. These results are consistent with data provided by Boston (1978) in studies involving controlled laboratory matings (at 25°C) of Palaemonetes pugio. The number of larvae hatching successfully from each brood was quite variable and the difference in mean hatch size between the two exposure groups was not statistically significant. A similarly high variation in hatch size was evident in the study by Boston (1978). In the present study, an effort was made to utilize female parents of consistent size in an attempt to minimize variations in egg-carrying capacity as related to her total length (Price, 1961; and Haeffner, 1972). There is no reason to doubt that such variability occurs in nature and that it may be influenced by such factors as: (1) the general health of the female, (2) the micro-environment of the oviducts and pleopods, (3) the fertilization success, (4) violent escape responses shortly after egg deposition, and (5) the period of time elapsed since the last brood was hatched (i.e. degree of ovarian development).

In an investigation of the euryhaline barnacle, Balanus amphitrite amphitrite, one group of eggs was incubated at a salinity of 15 ppt and another at 50 ppt (Crisp and Costlow, 1963). Larvae of the two groups that were exposed to low salinity (5-10 ppt) exhibited similar swimming behavior. When exposed to high salinities (50-60 ppt) after hatching a different response was observed. The high salinity-acclimated larvae swam normally but the low salinity-acclimated larvae became motionless within two hours. These results suggest the possible occurrence of a functional nongenetic adaptation to salinity. More meaningful conclusions may have been possible had the investigators used a more quantifiable measure of larval response.

Results of a laboratory study of the effects of embryonic and larval exposure to cadmium on salinity tolerance of larval Palaemonetes pugio, indicated no effect of pre-hatch exposure to salinity or 0.1 mg/l and 0.3 mg/l cadmium (Middaugh and Floyd, 1978). There was no significant decrease in survival of larvae (regardless of duration of embryonic exposure) when embryos were exposed to 30 ppt salinity and larvae reared at 10 ppt and 15 ppt salinity (no pre-hatch or post-hatch exposure to cadmium). There was, however, a noticeable effect of combined salinity/cadmium stress on survival--an indication that, as the authors point out, the stress of a pollutant may be more fully realized at sub-optimal salinities.

In that study, embryos were exposed to test conditions for, at most, eight days prior to hatching. This represents only approximately one-half of the total time required for embryonic development. Embryos in the present study were exposed from fertilization to hatching.

Rosenberg and Costlow (1979) investigated larval response to embryonic adaptation to salinity in the brachyuran crab, Rhithropanopeus harrissi Gould. In experiments comparable to those of the present study, the acclimation of embryos (in cyclic temperatures of 25°-30°C) at 10 ppt or 30 ppt had no statistically significant effect on subsequent larval survival, with one exception: all larvae hatched from embryos exposed to 30 ppt and reared at 5 ppt died in the first zoeal stage. Additional tests revealed another interesting phenomenon when embryos were exposed to 30 ppt for varying durations. The number of abnormal megalopae produced from larvae reared at 10 ppt, 20 ppt, and 30 ppt (25°-30°C) increased as a direct function of the number of days of embryonic exposure. Almost 80% of the larvae reared at 30 ppt developed abnormally at metamorphosis--essentially a delayed effect of acclimation and rearing at a stressful salinity. This process was shown to be, at least in part, reversible. If the period of embryonic acclimation was reduced to ten days or less, and larvae were reared at 10 ppt or 20 ppt, significantly fewer abnormal megalopae were produced.

Although the present study did not address the subject of temperature adaptation, Rosenberg and Costlow (1979) showed an effect of embryonic temperature adaptation at 35°C for Rhithropanopeus harrissi larvae. Larvae pre-adapted to two separate cyclic temperature regimes (25°-30°C and 30°-35°C) and reared at 35°C showed no survival to the first crab stage. However, larvae pre-adapted to a constant (stressful) temperature of 35°C and reared at that same temperature exhibited a 13% survival rate to the first crab stage.

Kinne (1953, 1958, 1962) has provided evidence for functional non-genetic adaptation to salinity of embryos of the euryhaline desert pupfish, Cyprinodon macularius. and the brackish-water amphipod, Gammarus duebeni. With C. macularius, it was found that fish hatched from eggs exposed to the test salinity and remaining at that salinity showed faster growth and more efficient food conversion than fish that were transferred to another salinity after hatching. It was suggested that this adaptation occurred within 3-6 hours following oviposition. Likewise, the salinity tolerance of G. duebeni was shown to depend upon the salinity in which fertilization and embryonic development occurred.

Results of this study indicate that an embryonic adaptation to salinity is possible in Palaemonetes pugio. The mechanism by which this occurs is not provided here. Burkenroad (1947) and Davis (1963) have presented

descriptions of crustacean hatching processes but did not address physiological and osmotic changes in the embryo throughout development. In studies of cirripede embryo, Crisp and Costlow (1963) suggested that the egg membrane is permeable to both water and substances of low molecular weight. They further suggest that embryonic adaptation may account for the successful dispersal and geographic range of Balanus amphitrite amphitrite. Pandian (1970), in an exhaustive analysis of embryonic development of the European lobster, Homarus gammarus, presented evidence that egg membranes are permeable to both water and salts but that this permeability varies throughout development as influenced by the egg's osmoconcentration. Results further indicated that the lobster egg absorbs water after fertilization increasing its water content to 59% by the time gastrulation is achieved and to 83% by the time hatching occurs. In addition to salt intake serving as an aid in the hatching process, the increasing imbibition of water through development offers an advantage with respect to exposure of the embryo to ambient water temperature.

Kinne (1964) describes adaptation as

> . . . an ecological phenomenon comprising adjustments of organisms to alterations in the intensity patterns of variables in their environment, which ultimately result in a relative increase in their capacity to survive, reproduce, or compete under the new conditions.

Organisms which are exposed to regular and extensive

variations in their physical and chemical environment are certainly more likely to develop the ability to adapt. Kinne (1962, 1963) further states that the ability of any organism to adapt may vary according to their stage of development and that this

> . . . capacity seems to reach its maximum during early ontogenetic development, that is, in eggs or early postnatal stages, and to decrease with increasing age of the individual.

In situations where it is desired to introduce an organism to a new environment (a common procedure in aquaculture practices and, on a smaller scale, transplants in nature) it may be advantageous to make this transfer during the development stage of maximum adaptability. The present study provides evidence in this light.

Both responses (larval survival and duration of development) are, undoubtedly, important from an ecological standpoint, with respect to the extent of larval distribution within an estuary and the degree to which larval dispersal plays in the geographic extension of Palaemonetes pugio populations. Other sub-lethal responses, less commonly studied, include swimming behavior, oxygen consumption, intermolt duration, number of instars attained at metamorphosis, and post-larval length. However, it is also important to bear in mind that conditions of temperature, salinity, food availability (both quantity and quality), light levels, predation, and physical disturbance are constantly changing, both spatially and temporally

within an estuary. The simple fact that Palaemonetes spp. have so successfully adapted to such a dynamic environment indicates that factor interaction is all-important, and it is to the net effect of this interaction, or to the total environmental 'picture,' that the individual is responding or acclimating. Widely varying results of laboratory rearing studies further cloud the concept of salinity optima in euryhaline species. Also complicating this scenario would be the differences in the ability of larvae at particular life history stages to tolerate stress induced by any one parameter. There is evidence that this phenomenon exists in crustaceans (Roberts, 1971; Sandifer, 1973; and Rosenberg and Costlow, 1979).

Studies, such as the one presented here, may indicate that seemingly similar laboratory rearing experiments (even involving the same species) may, in fact, utilize parental stocks with dissimilar environmental histories. Also, the collection of gravid females from field populations may introduce embryos which were spawned in different salinities. Little is known of the effect of ambient salinity at the discrete times of gamete formation, fertilization/oviposition, and hatching. Of particular significance in an estuarine environment is the concept of reversibility and irreversibility of the adaptation response as well as the effects of duration of embryonic exposure to specific factors or combinations of factors.

Chapter 5

RECOMMENDATIONS FOR FUTURE RESEARCH

It would be a rare case that the execution of a research project resulted in leaving the investigator convinced that the subject had been treated completely. As this thesis research progressed, several interesting questions arose. The following is a list of areas in which further investigation would aid in the understanding and interpretation of the results presented here:

1. The period of acclimation to test conditions may prove to play an important role in the success of early zoeal stages. Stepwise acclimation (both extent and duration) is a subject about which little is known.

2. Determination of the real limits of salinity tolerance of larvae of Palaemonetes pugio. Results of the present study indicate excellent rates of survival at 5 ppt, yet the results of other studies indicate very poor survival at low salinities.

3. In laboratory studies involving Crustacean larval rearing, Artemia salina nauplii are commonly utilized as the food organism. Work should be done to examine and quantify the effects of low salinity upon A. salina in a larval rearing environment.

4. Determine the effect (if any) of salinity at the discrete times of gamete formation, fertilization/oviposition, and hatching.

5. Determine if the adaptive responses indicated in this study are, in fact, reversible. Coupled with this analysis would be a study of the effects of the duration of embryonic exposure to sub-optimal conditions.

6. Investigate the interactive effects of temperature and salinity. Such factor interaction is known to be highly significant in laboratory studies and certainly in the natural environment.

7. Determine the extent to which inter-brood variation influences these results. The use of a large number of females treated similarly may reveal a significant parental effect. This would be of prime importance in understanding natural variability in patterns of larval development.

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APPENDIX A

SUMMARY OF ONE-WAY ANALYSIS OF VARIANCE FOR DIFFERENCES IN MEAN LARVAL SURVIVAL TO METAMORPHOSIS BETWEEN TWO GROUPS OF *Palaemonetes pugio* WITH DIFFERENT ADAPTIVE HISTORIES

Summary of one-way analysis of variance for differences in larval survival to metamorphosis for Palaemonetes pugio reared at three salinity levels. Analysis reflects arcsine transformation of percentage survival data.

Source of Variation	DF	SS	MS	Fs
CONTROL				
Among groups (rearing salinity)	2	1087.19	543.60	109.60*
Within groups (error; replicates)	9	44.62	4.96	-
Total	11	1131.81		
EXPOSED				
Among groups (rearing salinity)	2	102.23	51.12	1.42 ns
Within groups (error; replicates)	8	288.72	36.09	-
Total	10	390.95		

*P 0.001 (significant at 0.1% level)

ns = not significant

APPENDIX B

SUMMARY OF WITHIN-GROUP F-TESTS AND TESTS OF LEAST SIGNIFICANT DIFFERENCE SHOWING PAIRED COMPARISONS OF LARVAL SURVIVAL OF *Palaemonetes pugio* AT THREE REARING SALINITIES

Summary of within-group F-test and tests of least significant difference (unequal n) showing paired comparison of larval survival of Palaemonetes pugio at three rearing salinities.

Group	Comparison	SS	Fs	Significance Level
CONTROL	5‰ vs 10‰	269.0	54.23	0.001
	10‰ vs 20‰	274.6	55.36	0.001
	5‰ vs 20‰	1087.2	219.19	0.001
		Difference		
EXPOSED*	5‰ vs 10‰	7.53	ns	0.05
	10‰ vs 20‰	2.93	ns	0.05
	5‰ vs 20‰	4.60	ns	0.05

*Least significant differences = 9.25
ns = not significant

APPENDIX C

SUMMARY OF ONE-WAY ANALYSIS OF VARIANCE FOR DIFFERENCES IN LARVAL SURVIVAL TO METAMORPHOSIS FOR *Palaemonetes pugio* REARED AT THREE SALINITY LEVELS

Summary of one-way analysis of variance for differences in mean larval to metamorphosis between two groups of Palaemonetes pugio with different adaptive histories.

Between Groups	Mean % Survival (un-transformed)				
	Control	Exposed	SS[1]	MS	Fs
5‰	84.20	93.64	315.02	182.41	8.25*
10‰	95.75	98.25	127.86	51.68	3.39 ns
20‰	100.00	96.56	292.37	167.81	8.08*

[1]ANOVA performed on arcsine transformed percent survival data.

*P < 0.05

ns = not significant