1971

Larval Development of Pagurus longicarpus Say Reared in the Laboratory. II. Effects of Reduced Salinity on Larval Development

Morris H. Roberts Jr.
Virginia Institute of Marine Science

Reference: *Biol. Bull.*, **140**: 104–116. (February, 1971)

LARVAL DEVELOPMENT OF *PAGURUS LONGICARPUS* SAY REARED IN THE LABORATORY. II. EFFECTS OF REDUCED SALINITY ON LARVAL DEVELOPMENT[1]

MORRIS H. ROBERTS, JR.[2]

Virginia Institute of Marine Science, Gloucester Point, Virginia 23062

Temperature and salinity define a set of conditions within which planktonic organisms can survive and develop. Thorson (1946) described the restriction of some meroplankters to Kattegat water in the Oresund which was presumed to be based on either a temperature, or a salinity discontinuity or both. Bary (1963a, b, c), in an extensive study of North Atlantic plankton, clearly demonstrated a relationship between zooplankton distribution and temperature-salinity distribution. Banse (1956) observed the distribution of polychaete and echinoderm larvae with respect to various water masses in Kiel Bay. He concluded that these larvae were restricted to their "Gebirtswasser" by the temperature-salinity characteristics of these water masses. In a subsequent paper (1959) he described a similar situation for copepods.

Survival and rate of development of decapod larvae are temperature-dependent phenomena. It has been shown for a variety of species that there is some optimal temperature range above and below which larval mortality increases (Boyd and Johnson, 1963; Chamberlain, 1961, 1962; Costlow, 1967; Costlow and Bookhout, 1962, 1968; Costlow, Bookhout and Monroe, 1960, 1962, 1966; Coffin, 1958, 1960). There is a unique range of temperature permitting survival for each decapod species so far studied. Further, it has been demonstrated that intermolt duration decreases with increasing temperature.

While temperature affects animal distributions both in the open sea and coastal waters, salinity has the greatest influence in coastal waters and estuaries. The ability of larvae to survive reduced salinity is therefore of extreme interest in the case of estuarine species.

Considerable research has been conducted to elucidate the effects of salinity on larvae of estuarine brachyurans (Chamberlain, 1961, 1962; Costlow, 1967; Costlow and Bookhout, 1962, 1968; Costlow *et al.*, 1960, 1962, 1966). Salinity strongly affects survival with tolerance ranges unique for each developmental stage and each species. There seems to be little effect on intermolt duration until the lethal salinity is neared, at which point a slight increase can be detected. Information is available for only three anomuran species, *Pisidia longicornis* (Lance, 1964),

[1] Contribution Number 368 from the Virginia Institute of Marine Science, Gloucester Point, Virginia 23062.

[2] Present address: Department of Biology, Providence College, Providence, Rhode Island 02918. This paper is part of a dissertation submitted to the School of Marine Science of The College of William and Mary in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

Pagurus samuelis (Coffin, 1958) and *Pagurus bernhardus* (Bookhout, 1964). The general trends are the same as for brachyurans.

In the present study the effects of reduced salinity on embryos and larvae of *Pagurus longicarpus* were determined. Efforts were made to detect changes in salinity tolerance with increasing developmental age.

Despite the importance of temperature in larval development, this parameter was not included as a variable in these studies. Limitations of equipment and time made it impractical to conduct experiments at several temperatures. While there may well be important interaction effects of salinity and temperature, it did not seem reasonable to pursue this aspect of the problem at this time.

The four zoeae and megalopa of *Pagurus longicarpus* were described in an earlier paper (Roberts, 1970). It was reported that these larvae did not correspond to those described by Thompson (1903) as *P. longicarpus*. It was suggested that Thompson had been working with *P. annulipes*. Nyblade (1970), based on his study of the larvae of the latter species, concluded that Thompson was indeed studying *P. annulipes*.

Materials and Methods

Embryological development

Information on embryological development was obtained incidentally to the culture of eggs for various experiments. Eggs removed from the pleopods of a single female were incubated in filtered water at several salinities following the procedures developed by Costlow and Bookhout (1960). Quantitative observations, such as per cent hatch, were not attempted, but it was noted if development and hatching occurred and if there were any delay in hatching.

Larval development

Larvae for these experiments were hatched from females maintained in 5-liter battery jars containing water of 20‰ salinity. The water was replaced daily. Preliminary studies showed that eggs did not develop at all salinities included in this study. Further, no consistent difference was detected between mortality of larvae hatched at salinities from 15 to 30‰ *versus* larvae hatched at 20‰ and subsequently transferred to water with salinities from 15 to 30‰. Larvae were reared in water of 20‰ on a diet of nauplii of *Artemia* to the desired zoeal stage for each experiment. Tolerance tests were conducted in small finger bowls containing 200 ml water, 10 larvae per bowl. Fifty larvae were subjected to each test salinity in the range 10 to 30‰. All experiments were conducted at 20 ± 2° C, a temperature intermediate in the range tolerated by these larvae, though not necessarily optimal. This temperature is somewhat below the mean summer temperature in the York River estuary, but was convenient for the present study.

Four experiments of the above design were run, each experiment beginning with a different developmental stage. The experiment number corresponded with the zoeal stage used at the start of the test; thus Experiment 1 began with Zoea I, Experiment 2 with Zoea II and so forth. It was hoped in this manner to detect any increase (or decrease) in ability to tolerate reduced salinity as a function of

zoeal stage and to detect any effect of prior culture conditions at the test salinity on each stage.

In the four experiments described above, tests were terminated after the molt to the megalopa since in most cases too few larvae reached this stage in healthy condition to produce meaningful results. An independent test, Experiment 5, was conducted with megalopae obtained from mass culture (100–200 larvae in 1000 ml, 20‰, 20° C). Since megalopae are aggressive and, in mass culture, frequently kill one another, compartmented boxes were used with one megalopa per compartment (50 ml). The same series of salinities was used as for tests with zoeae.

TABLE I

Hatching time as a function of incubation salinity and degree of development at start of incubation

Initial degree of development	Temperature (°C)	Days to hatching	
		20 to 30‰	15‰
Eyespot*	30	2	2
Eyestreak**	30	3	3
Eyestreak†	25	4	4
½ yolk***	25	5	6
"Early"****	25	7	9
"Early"	25	7	8

* Eyespot refers to eggs with dark brown or black circular eyespots and a beating heart.
** Eyestreak refers to eggs in which the eye is represented by a brown crescent-shaped spot.
*** ½ yolk refers to eggs with yolk occupying about ½ the egg capsule.
**** "Early" refers to eggs with at most a small white germinal disc apparent.
Further details concerning these stages are given in Coffin (1960).
† Artificial sea water (Rila mix) used in this experiment.

Water for all tests was prepared by diluting high salinity water collected at the branch laboratory at Wachapreague, Virigina. Water was collected from Finney Creek, filtered through a 1 μ filter, passed beneath ultraviolet lights, and collected in carboys. After transit to the main laboratory at Gloucester Point, the water was stored in large darkened carboys until used.

RESULTS

Embryological development

Development of embryos was followed at salinities of 10, 15, 20, 25 and 30‰. The results are summarized in Table I. Embryonic development was successfully completed at all salinities except 10‰ at which development proceeded for one or two days after which the yolk broke down. The eggs were greatly swollen and deteriorated rapidly. The same result was obtained even with late-stage eyed embryos placed in water of 10‰ salinity. Viable larvae hatched at 15‰ but generally 24–48 hours later than those hatched at higher salinities. The delay in development was more pronounced if eggs in an early stage of development were used. No effect was detected at salinities from 20 to 30‰.

Larval development: Survival

In all experiments, many deaths occurred just before, during, or immediately after a molt, especially the fourth molt. Larvae dying just prior to a molt showed anlagen of structures present in the succeeding stage beneath the old integument and separation of hypodermis from exuvium. These larvae apparently could not split the old integument, or could not remove themselves from it before they swelled. Larvae dying during the molt swelled while partly out of the exuvium. These larvae, with abdomen and part of the cephalothorax removed from the exuvium, were recorded as the stage following the molt. At the lowest salinities, the dorsum of the carapace was sometimes shed before the abdomen; these larvae were classed as the stage prior to the molt. Those dying immediately after the molt usually appeared weak at the last observation before death, and may have succumbed from the effort of completing the molt.

In Experiment 1, complete development to the megalopa was observed at all salinities from 15.5 to 30.5‰. No significant difference in survival was noted from 18.0 to 30.5‰ with 25 to 60% of the larvae reaching the megalopa. At 15.5‰, only 8% reached the megalopa. At 13.0‰ about 70% molted to Zoea II and 10% to Zoea III, but none to Zoea IV. At 10.5‰ only 2% survived to Zoea II and none to Zoea III (Fig. 1a). At salinities from 15.5 to 30.5‰, the slope of the survivorship curve was relatively constant up to Zoea IV but showed a marked increase for Zoea IV. At 13.0‰ there was a sharp increase in mortality for Zoea II.

Mortality observed immediately following transfer to 10.5 and 13.0‰ was due to the low salinity, not the salinity difference involved. Larvae transferred from 20.5 to 30.5‰, a salinity difference of equal magnitude, though in the opposite direction, did not exhibit significant mortality immediately after transfer. Further, 1 or 2 days after the larvae had been transferred to 10.5 and 13.0‰, mortality dropped to zero, and remained at zero until the next molt which was considerably delayed (see below).

Observations of larval development at salinities of 18.0 and 13.0‰ were omitted from the series in Experiment 2, but the results were essentially the same as in Experiment 1 with complete development to the megalopa observed from 15.5 to 30.5‰. From 20.5 to 30.5‰, 35 to 55% survived to the megalopa; at 15.5‰, only 4%. No larvae reached Zoea III at 10.5‰. Most larvae in this latter salinity died shortly after transfer, the remainder just prior to the molt. The slopes of the survivorship curves are essentially constant up to Zoea IV at all salinities except 10.5‰ (Fig. 1b).

Complete development occurred over the salinity range 13.0 to 30.5‰ in Experiment 3. From 18.0 to 30.5‰, 60 to 83% reached the megalopa, at 15.5‰, 40% and at 13.0‰, 5% (Fig. 1c). Thus at 15.5‰ there was an apparent increase in survival to the megalopa from 4–8% to 40% while at 13.0‰, the increase is from 0% to 5%.

Megalopae were obtained in every salinity including 10.5‰ in Experiment 4. Better than 80% survived at all salinities from 13.0 to 30.5‰, and 5.3% at 10.5‰ (Fig. 1d).

While it might seem that there is a marked increase in salinity tolerance with increasing developmental age, a comparison of the family of survivorship curves

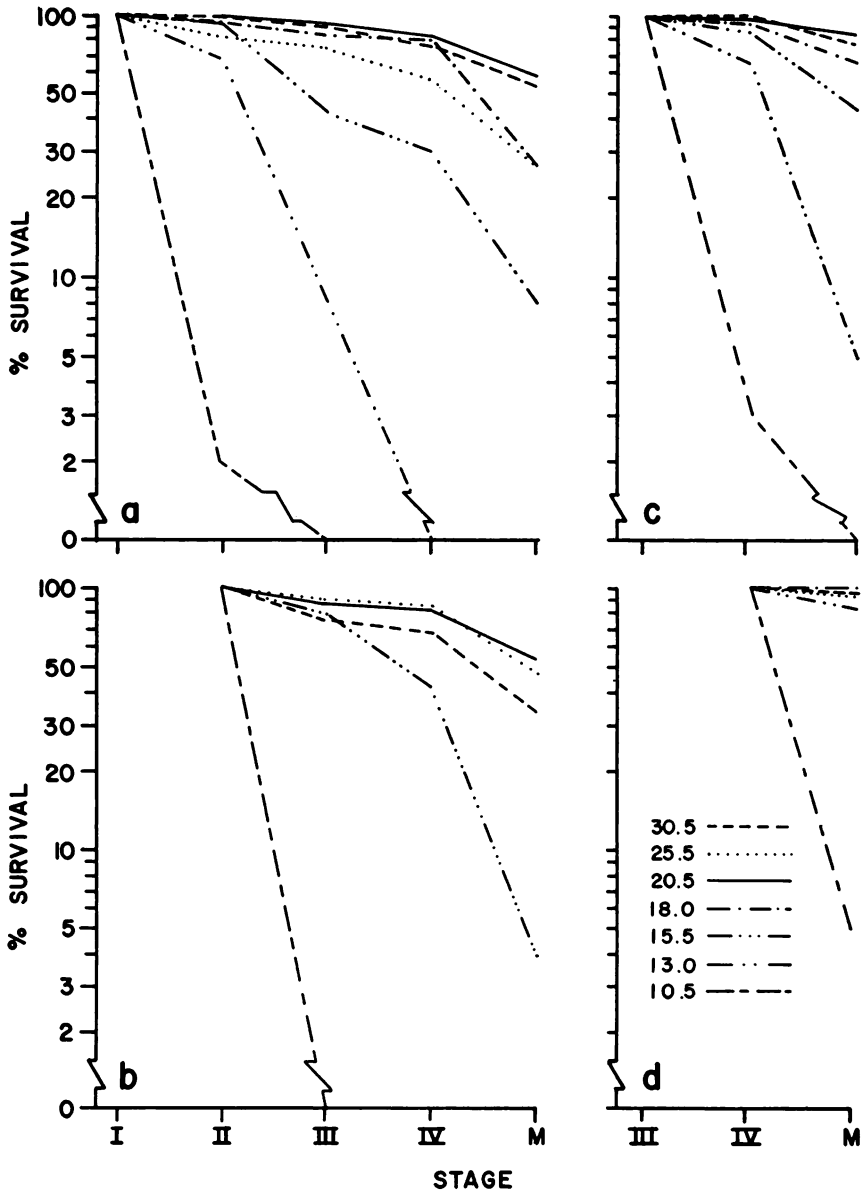

FIGURE 1. Survivorship curves for the salinity tolerance experiments; (a) Experiment 1, (b) Experiment 2, (c) Experiment 3, (d) Experiment 4. In Experiment 4, the results were identical for 30.5 and 20.5 ‰ and for 18.0 and 15.5 ‰.

for the four experiments suggests that this is not the case. The curves for each experiment are exactly like those of Experiment 1 if the latter are displaced an appropriate number of developmental stages to the right.

In order to compare the mortality in each zoeal instar in the various experiments, the per cent mortality was calculated as follows:

$$\% M = \frac{n_i - n_{i+1}}{n_i} \times 100$$

where

n_i = number of Zoea i
n_{i+1} = number of Zoea $i + 1$

These values are arrayed in Table II. Assuming that the per cent mortalities so obtained are binomially distributed, it is possible to estimate 95% confidence limits from tables for the appropriate sample size and point estimate (Diem, 1962). From a consideration of the 95% confidence limits and the point estimates for each cell in the array in Table II, I was led to the conclusion that three values were not adequate estimates of the mortality resulting from salinity. These values are enclosed in brackets.

The initial zoeal stage used in each experiment had a lower per cent mortality than succeeding stages. Indeed, no difference could be detected in the per cent mortality of the initial stage of each experiment at any given salinity. This can be seen by comparing the per cent mortality for the initial stage used in each experiment at any given salinity in Table II. This results from the selection of only healthy larvae, *i.e.,* those showing a strong positive light response, in setting up each experiment. The increase in mortality in succeeding stages, which usually was not very great except in Zoea IV, reflects at least in part the fact that some larvae remaining after the initial molt were not healthy.

There was no significant difference in per cent mortality for any given zoeal stage over the salinity range 18.0 to 30.5‰. This can be seen by comparing values for any given stage, *e.g.,* Zoea III, over the entire range of salinity. Per cent mortality for the initial stage in each experiment at 15.5‰ was in the same range as that of 18.0 to 30.5‰, but many larvae were weakened as indicated by the significant increase in mortality in the succeeding stage. The same effect was noted at 13.0‰ but at a significantly higher mortality level. If tolerance of reduced salinity had improved with increasing developmental age, one would expect a marked improvement in survival of later stages in 15.5‰ in the later experiments.

Per cent mortality for the megalopa (Experiment 5) was less than 6% from 18.0 to 30.5‰, which agrees with the results obtained for the zoeal stages. At lower salinities per cent mortality increased rapidly to 100% at 10.5‰. At 15.5‰ the per cent mortality was significantly higher than at higher salinities with most deaths occurring near the time of molt to the juvenile. Deaths at 13.0‰ also were associated with molting, while those at 10.5‰ were associated with the stress produced by transfer to this salinity. This may represent a slightly lessened ability of the megalopa to tolerate reduced salinity.

Larval development: Intermolt duration

The mean time after hatching to each zoeal stage, in days, was calculated for all experiments. The number molting during each 24-hour period was asso-

TABLE II

Per cent mortality for the zoeal stages and megalopae at each salinity

Salinity	Experiment number	Zoeal stages				
		I	II	III	IV	Megalopa
30.5	1	2.0	8.2	15.5	31.4	/
	2	—	[21.6]	12.8	50.0	/
	3	—	—	0.0	22.5	/
	4	—	—	—	2.6	/
	5	—	—	—	—	5.6
25.5	1	[18.0]	9.8	[24.3]	51.8	/
	2	—	10.0	6.7	57.2	/
	3	—	—	—	—	/
	4	—	—	—	5.3	/
	5	—	—	—	—	0.0
20.5	1	2.0	7.1	11.0	29.0	/
	2	—	14.0	4.7	34.9	/
	3	—	—	2.5	15.4	/
	4	—	—	—	2.6	/
	5	—	—	—	—	2.8
18.0	1	6.0	10.6	4.8	67.5	/
	2	—	—	—	—	/
	3	—	—	5.0	31.6	/
	4	—	—	—	0.0	/
	5	—	—	—	—	5.6
15.5	1	7.0	54.8	28.6	73.5	/
	2	—	20.0	47.6	90.5	/
	3	—	—	15.0	50.0	/
	4	—	—	—	0.0	/
	5	—	—	—	—	22.2
13.0	1	32.0	88.3	100.0	/	/
	2	—	—	—	—	/
	3	—	—	35.0	92.0	/
	4	—	—	—	15.8	/
	5	—	—	—	—	66.7
10.5	1	98.0	100.0	/	/	/
	2	—	100.0	/	/	/
	3	—	—	97.6	100.0	/
	4	—	—	—	94.7	/
	5	—	—	—	—	100.0

ciated with the mean time after hatching for that period for the purposes of calculation. This procedure assumes that the frequency of molting was normally distributed through time.

Mean post-hatching time, range of post-hatching time, and number of larvae involved for each zoeal stage are presented in Table III and Figure 2. Intermolt duration for each zoeal stage was then calculated as the difference between the

mean post-hatching times from Experiment 1, except in cases where no information was available. When information was available from the other experiments, it was used instead.

Rigorous statistical comparison of mean post-hatching time for each molt was not possible because the observation intervals were very large relative to the interval between the first and last zoea completing a given molt. Such intensive grouping causes a gross underestimate of the true variance, thereby increasing the chance of rejecting the null hypothesis that the mean post-hatching times were equal, when it should be accepted. Intensive grouping also tends to bias the

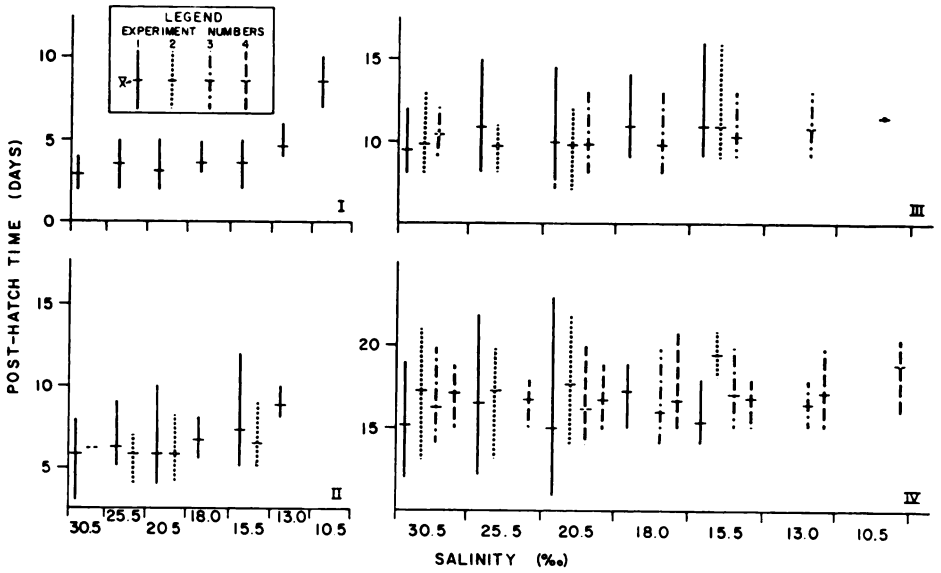

FIGURE 2. Range and mean post-hatch time for each zoeal stage.

estimate of the mean in an indeterminate manner since there is no way of knowing in any given case whether the larvae molted at the beginning or end of an observation period.

The intermolt duration over the salinity range 15.5 to 30.5‰ was 3.0 to 3.5 days for Zoea I, 2.7 to 3.8 days for Zoea II, 3.5 to 4.6 days for Zoea III, 4.6 to 6.4 days for Zoea IV. Intermolt duration increased slightly at 13.0 and 10.5‰.

Mean post-hatching times derived from Experiments 1 to 4 show very close agreement at each salinity except in some cases for the molt from Zoea IV to megalopa (Fig. 2). The poor agreement in this case results in part from the small numbers of larvae involved.

Intermolt duration for the megalopa was obtained directly since the protocol for this experiment was slightly different. The intermolt duration was 6.5 to 7.5 days at salinities from 18.0 to 30.5‰, increasing to 8 and 9 days at 15.5 and 13.0‰, respectively. The increased intermolt duration for Zoea IV and the

TABLE III

Mean post-hatching times to the end of each zoeal stage and intermolt durations for each larval stage

Developmental stage	Experiment number		Salinity, ‰ 30.5	25.5	20.5	18.0	15.5	13.0	10.5
Zoea I	1	x̄	2.95	3.50	3.04	3.52	3.54	4.59	8.50
		r	(2–4)	(2–5)	(2–5)	(3–5)	(2–5)	(4–6)	(7–10)
		n	49	41	98	47	93	34	2
	Intermolt duration		3.0	3.5	3.1	3.5	3.5	4.6	8.5
Zoea II	1	x̄	5.88	6.23	5.82	6.60	7.31	8.75	—
		r	(3–8)	(5–9)	(4–10)	(6–8)	(5–12)	(8–10)	—
		n	45	37	91	42	42	4	—
	2	x̄	6.04	5.81	5.76	—	6.40	—	—
		r	(4–8)	(4–7)	(4–8)	—	(5–9)	—	—
		n	39	45	43	—	40	—	—
	Intermolt duration		2.9	2.7	2.7	3.1	3.8	4.2	—
Zoea III	1	x̄	9.50	10.81	9.94	10.95	10.80	—	—
		r	(8–12)	(8–15)	(7–14)	(9–14)	(9–16)	—	—
		n	38	28	81	40	30	—	—
	2	x̄	9.85	9.67	9.70	—	10.83	—	—
		r	(8–13)	(8–11)	(8–12)	—	(9–16)	—	—
		n	34	42	41	—	21	—	—
	3	x̄	10.40	—	9.81	9.71	10.24	10.81	11.50
		r	(9–12)	—	(8–13)	(8–13)	(9–13)	(9–13)	(12)
		n	40	—	39	38	34	26	1
	Intermolt duration		3.6	4.6	4.1	4.4	3.5	(5.0)	(5.7)
Zoea IV	1	x̄	15.12	16.58	15.01	17.35	15.38	—	—
		r	(12–19)	(12–22)	(11–23)	(15–19)	(14–18)	—	—
		n	26	13	58	13	8		
	2	x̄	17.26	17.19	17.69	—	19.50	—	—
		r	(13–21)	(13–20)	(14–22)	—	(18–21)	—	—
		n	17	24	27	—	2	—	—
	3	x̄	16.40	—	16.17	16.04	17.09	16.50	—
		r	(14–20)	—	(14–20)	(14–20)	(15–20)	(15–18)	—
		n	31	—	33	26	17	2	—
	4	x̄	17.12	16.78	16.82	16.74	16.84	17.09	19.00
		r	(15–19)	(15–18)	(15–19)	(15–21)	(15–18)	(15–20)	(16–21)
		n	37	36	37	38	38	32	2
	Intermolt duration		5.6	5.8	5.1	6.4	4.6	(5.7)	(8.1)
Megalopa*	5	x̄	7.35	6.47	6.55	7.04	8.18	9.08	—
		r	(6–8)	(5–8)	(5–9)	(5–9)	(6–10)	(7–11)	—
		n	34	36	35	34	28	12	—

* Values for this stage are given as intermolt duration, in days, rather than post-hatching time, in days, as for all other stages.

megalopa compared to earlier zoeal stages has been noted before in *Petrochirus diogenes* (Provenzano, 1968).

Discussion

The notion that certain life history stages are more subject to limitation by abiotic environmental factors such as salinity, was first enunciated by Shelford (1915). Since then, considerable evidence has been amassed demonstrating that younger stages tend to be less tolerant than adults. Among decapod crustaceans, perhaps the best documented example is the blue crab, *Callinectes sapidus,* which can tolerate salinities from fresh to oceanic as an adult, but must return to water with a salinity in excess of 15‰ for hatching of the eggs. Complete larval development occurs only at 20‰ and above (Sandoz and Rogers, 1944; Costlow and Bookhout, 1959; Costlow, 1967). Other examples from various phyla may be found in recent reviews by Kinne (1964, 1966) and other papers in the literature.

Salinity tolerance of decapod embryos has received only cursory attention. Broekhuysen (1936) cultured *Carcinus maenas* eggs at salinities from 10 to 50‰. At salinities from 20 to 40‰, 16° C, and 25 to 40‰, 10° C, complete embryonic development occurred, while at salinities above and below this range, development occurred to a degree, but hatching was not observed. At 10‰, no embryonic development was detected. Tolerance of each larval stage is unknown, but the adult tolerance is from 4 to 34‰ salinity (or above). Hatching of *Hepatus epheliticus* has been observed at all salinities from 20 to 40‰ which is a greater range than tolerated by either larvae or adults (Costlow and Bookhout, 1962). In the present study, hatching of *P. longicarpus* was observed from 15 to 30‰ at 25 and 30° C. This is very close to the larval tolerance but probably slightly less than the adult tolerance, although the latter is not precisely known.

It is concluded that each species has a unique range of salinities suitable for embryonic development and hatching which bears no *a priori* relationship to the tolerance of adults or larvae. The egg is by no means the most sensitive life history stage in all decapod crustaceans.

Salinity tolerances for the larvae of several brachyuran species have now been studied in detail, in several cases in combination with temperature tolerances. *Rhithropanopeus harrisii* can develop over the salinity range 1 to 40‰ with optimal development between 15 and 30‰ at 20 to 30° C (Chamberlain, 1962; Costlow *et al.*, 1966). However, field observations from the Miramichi Estuary indicate that *Rhithropanopeus* larvae are usually restricted to water with a salinity less than 23.5‰ (Bousfield, 1955). This probably reflects the distribution of the adults which is similarly restricted. *Sesarma cinereum* has a different optimal salinity range for each larval stage, with Zoea IV most sensitive (optimum: 15–25‰, 30° C, 22–28‰, 20° C). The total range tolerated is much greater than the optimal range (Costlow *et al.*, 1960). *Panopeus herbstii* larvae tolerate a broader salinity range than *S. cinereum,* but not as broad a range as *R. harrisii.* In *Panopeus,* no stage is particularly sensitive to salinity (Costlow *et al.*, 1962). *Callinectes,* an estuarine species like those above, was mentioned previously in this regard. *Hepatus epheliticus,* a polystenohaline crab, develops completely over

only a narrow salinity range of 30 to 35‰ (Costlow and Bookhout, 1962). The terrestrial brachyuran, *Cardisoma guanhumi,* exhibits complete development from 15 to 45‰. The special adaptive osmoregulatory mechanisms of the adult are not developed in the larvae, which have a tolerance range comparable to an estuarine species (Costlow and Bookhout, 1968).

Lance (1964) used a different procedure for studying tolerance levels of *Carcinus maenas* Zoea I and megalopa and *Pisidia longicornis* Zoea I and II and megalopa. She transferred larvae to containers with water of varying salinities and counted the number dead after 20 hours, at which time the experiment was terminated. *Carcinus maenas* Zoea I was less tolerant than the megalopa, with 50% mortality at salinities below 12.6 and 9.9‰ respectively. The two zoeal stages of *Pisidia longicornis* were identical in tolerance capacity with more than 50% mortality at salinities below 14.4‰. The megalopa is less tolerant, with more than 50% mortality at salinities below 20.7‰.

Lance (1964) measured the acute effects of salinity during the intermolt period rather than the chronic effects of prolonged exposure to various salinities. It has been observed by many investigators that most deaths attributable to salinity occur about the time of ecdysis unless the salinity is extreme relative to the tolerance ability of the given species. At ecdysis, major changes in the cuticle in effect lower the defenses of the animal. Tolerance ranges based on studies of acute salinity effects tend to be broader than the actual tolerance range. To understand the effect of salinity on distribution, one must know the true tolerance range based on studies of chronic effects of salinity.

Of the pagurid species studied previously, *Pagurus samuelis* was observed to develop to Zoea IV only from 22.5 to 35‰, and to the megalopa only at 29‰. No juveniles were obtained in salinity tolerance experiments because the temperature exceeded the lethal limit late in the experiment (Coffin, 1958). *Pagurus bernhardus* developed completely to the juvenile only at 30 and 35‰. A small percentage of megalopae were obtained at 25‰ but this result was not duplicated in another experiment (Bookhout, 1964).

Pagurus longicarpus developed over a much wider salinity range than either *Pagurus* species mentioned above. Of the three species, this is the only one penetrating estuaries to a significant extent. The optimal salinity range is broad, at least from 18 to 30.5‰ and probably above. Over this range there was no detectable difference in mortality or intermolt duration. Tolerance of reduced salinity is the same for all four zoeal stages, slightly less for the megalopa. The optimal salinity range is only slightly less than the total salinity range permitting complete development and is one of the broadest optimal salinity ranges known.

Coffin (1958) indicated the possibility of cannibalism during zoeal stages in his experiments. Bookhout (1964) also concluded there was cannibalism, as much as 15 to 35% at 35‰, decreasing to 2.5 to 27.5% at 25‰ in his experiments. In the present study, no evidence of cannibalism has been observed during the zoeal stages even in large mass cultures with larval densities of 1 larva per 5 ml. Maximum density in the tolerance experiments was only 1 larva per 20 ml.

Cannibalism has been observed among megalopae in mass culture, necessitating rearing them in isolation. Thompson (1903) and Bookhout (1964) reported high mortality rates for megalopae not provided with shells and low mortality

rates for those with shells. Since both authors reared larvae in mass cultures, megalopae with shells were less likely to be cannibalized than those without. In addition, Bookhout (1964) was dealing with sample sizes too small to draw very firm conclusions. In an independent set of experiments to be described in a subsequent paper, no difference was observed in mortality rate of megalopae provided with shells and those without when the tests were conducted with each megalopa isolated in compartmented boxes. The results of the experiment on salinity tolerance of megalopae are therefore not subject to criticism because no shells were provided for the larvae.

It is not possible to compare in detail larval and adult tolerances for *P. longicarpus*, as the latter have not been studied. However, it is known that for the adults, the haemolymph has the same osmotic concentration as the medium over the salinity range 18 to 43‰ (Kinne, Shirley and Meen, 1963).

I am indebted to Dr. Langley Wood, who served as chairman of my graduate committee, for his patience and helpful criticisms. I also appreciate the many fruitful discussions with Dr. Marvin L. Wass and Mr. Willard A. Van Engel and their critical reading of the manuscript. Dr. Morris L. Brehmer provided space in his laboratory and a critical review of the manuscript. Dr. Edwin Joseph provided a constant temperature water bath. I am very grateful to Mrs. Jane S. Davis and Mrs. K. Stubblefield for their patient aid in preparing the figures.

I owe special thanks to my wife, Beverly Ann, for her constant encouragement and aid in the laboratory.

During the course of this study I was the recipient of a National Science Foundation Graduate Fellowship.

LITERATURE CITED

BANSE, K., 1956. Über den Transport von meroplanktischen Larven aus dem Kattegat in die Kieler Bucht. *Ber. Deut. Wiss. Komm. Meeresforsch.*, **14**: 147–164.

BANSE, K., 1959. Die Vertikalverteilung planktischer Copepoden in der Kieler Bucht. *Ber. Deut. Wiss. Komm. Meeresforsch.*, **15**: 357–388.

BARY, B. M., 1963a. Temperature, salinity and plankton in the eastern North Atlantic and coastal waters of Britain, 1957. I. The characterization and distribution of surface waters. *J. Fish. Res. Board, Can.*, **20**: 789–826.

BARY, B. M., 1963b. Temperature, salinity and plankton in the eastern North Atlantic and coastal waters of Britain, 1957. II. The relationships between species and water bodies. *J. Fish. Res. Board, Can.*, **20**: 1031–1065.

BARY, B. M., 1963c. Temperature, salinity and plankton in the eastern North Atlantic and coastal waters of Britain, 1957. III. The distribution of zooplankton in relation to water bodies. *J. Fish. Res. Board, Can.*, **20**: 1519–1548.

BOOKHOUT, C. G., 1964. Salinity effects on the larval development of *Pagurus bernhardus* (L.) reared in the laboratory. *Ophelia*, **1**: 275–294.

BOUSFIELD, E. L., 1955. Ecological control of the occurrence of barnacles in the Miramichi Estuary. *Bull. Nat. Mus. Canada*, **137**: 1–69.

BOYD, C. M., AND M. W. JOHNSON, 1963. Variations in the larval stages of a decapod crustacean, *Pleuroncodes planipes* Stimpson (Galatheidae). *Biol. Bull.*, **124**: 141–152.

BROEKHUYSEN, G. J., JR., 1936. On development, growth, and distribution of *Carcinides maenas* (L.). *Arch. Neerl. Zool.*, **2**: 257–399.

COFFIN, H. G., 1958. The laboratory culture of *Pagurus samuelis* Stimpson. *Walla Walla Coll. Publ.*, **22**: 1–5.

COFFIN, H. G., 1960. The ovulation, embryology, and developmental stages of the hermit crab, *Pagurus samuelis* Stimpson. *Walla Walla Coll. Publ.*, **25**: 1–30.

CHAMBERLAIN, N. A., 1961. Studies on the larval development of *Neopanope texana sayi* (Smith) and other crabs of the Zanthidae (Brachyura). *Chesapeake Bay Institute, Johns Hopkins Univ., Tech Rep.*, **22**: 1–35.

CHAMBERLAIN, N. A., 1962. Ecological studies of the larval development of *Rhithropanopeus harrisii* (Xanthidae, Brachyura). *Chesapeake Bay Institute, Johns Hopkins Univ., Tech. Rep.*, **28**: 1–47.

COSTLOW, J. D., JR., 1967. The effect of salinity and temperature on survival and metamorphosis of megalops of the blue crab, *Callinectes sapidus* Rathbun. *Helgolaender Wiss. Meeresunters.*, **15**: 84–97.

COSTLOW, J. D., JR., AND C. G. BOOKHOUT, 1959. The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. *Biol. Bull.*, **116**: 373–396.

COSTLOW, J. D., JR., AND C. G. BOOKHOUT, 1960. A method for developing Brachyuran eggs *in vitro*. *Limnol. Oceanogr.*, **5**: 212–215.

COSTLOW, J. D., JR., AND C. G. BOOKHOUT, 1962. The larval development of *Hepatus epheliticus* (L.) under laboratory conditions. *J. Elisha Mitchell Sci. Soc.*, **78**: 113–125.

COSTLOW, J. D., JR., AND C. G. BOOKHOUT, 1968. The effect of environmental factors on the development of the land-crab *Cardisoma guanhumi* Latreille. *Amer. Zool.*, **8**: 397–410.

COSTLOW, J. D., JR., C. G. BOOKHOUT AND R. MONROE, 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. *Biol. Bull.*, **118**: 183–202.

COSTLOW, J. D., JR., C. G. BOOKHOUT AND R. MONROE, 1962. Salinity-temperature effects on the larval development of the crab, *Panopeus herbstii* Milne-Edwards, reared in the laboratory. *Physiol. Zool.*, **35**: 79–93.

COSTLOW, J. D., JR., C. G. BOOKHOUT AND R. MONROE, 1966. Studies on the larval development of the crab, *Rhithropanopeus harrisii* (Gould). I. The effect of salinity and temperature on larval development. *Physiol. Zool.*, **39**: 81–100.

DIEM, K. (Ed.), 1962. *Documenta Geigy, Scientific Tables.* [6th Edition] Geigy Pharmaceuticals, Ardsley, New York, 778 pp.

KINNE, O., 1964. The effects of temperature and salinity on marine and brackish water animals. II. Salinity. *Oceanogr. Mar. Biol., Ann. Rev.*, **2**: 281–339.

KINNE, O., 1966. Physiological aspects of animal life in estuaries with special reference to salinity. *Netherlands J. Sea Res.*, **3**: 222–244.

KINNE, O., E. K. SHIRLEY AND H. E. MEEN, 1963. Osmotic responses of hermit crabs (*Pagurus longicarpus* Say) exposed to various constant temperatures and salinities. *Crustaceana*, **5**: 317.

LANCE, J., 1964. The salinity tolerances of some estuarine planktonic crustaceans. *Biol. Bull.*, **127**: 108–118.

NYBLADE, C., 1970. Larval development of *Pagurus annulipes* (Stimpson, 1862) and *Pagurus pollicaris* Say, 1817 reared in the laboratory. *Biol. Bull.*, **139**: 557–573.

PROVENZANO, A. J., JR., 1968. The complete larval development of the West Indian hermit crab *Petrochirus diogenes* (L.) (Decapoda, Diogenidae) reared in the laboratory. *Bull. Mar. Sci.*, **18**: 143–181.

ROBERTS, M. H., JR., 1970. Larval development of *Pagurus longicarpus* Say reared in the laboratory, I. Description of larval instars. *Biol. Bull.*, **139**: 188–202.

SANDOZ, M., AND R. ROGERS, 1944. The effect of environmental factors on hatching, moulting, and survival of the zoea larvae of the blue crab, *Callinectes sapidus* Rathbun. *Ecology*, **25**: 216–228.

SHELFORD, V. E., 1915. Principles and problems of ecology as illustrated by animals. *J. Ecol.*, **3**: 1–23.

THOMPSON, M. T., 1903. The metamorphosis of the hermit crab. *Proc. Boston Soc. Natur. Hist.*, **31**: 147–209.

THORSON, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates. *Medd. Dan. Fisk. Havunders*, **4**: 1–523.