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THE EFFECT OF SALINITY AND TEMPERATURE ON THE LARVAL DEVELOPMENT OF *CLIBANARIUS VITTATUS* (Bosc) (CRUSTACEA: DECAPODA: DIOGENIDAE)[1]

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Abstract: *Clibanarius vittatus* (Bosc) larvae were reared in twenty combinations of four salinities (15, 20, 25, and 30‰) and five temperatures (15, 20, 25, 30, and 35 °C). No development was observed in any salinity at 15 °C, but partial development occurred in all other test conditions. Metamorphosis to juvenile crabs was noted only at salinities of 25 and 30‰ in combination with temperatures of 25 and 30 °C. In general, development times were decreased at higher temperatures; no trend was evident for salinity. Mortality of zoeae was usually highest at the time of the first molt and greatest overall mortality occurred during the megalopa stage prior to metamorphosis. Previous experiments (unpubl.) have shown that *C. vittatus* adults can tolerate temperatures down to 5 °C. It is suggested that the geographic distribution of *C. vittatus* (Virginia, southwards) is limited not by adult tolerances but by the inability of the species to establish a breeding population. Larvae require two months at 25 °C or above to metamorphose, and this condition is not met in areas north of Virginia.

INTRODUCTION

It has long been recognized that salinity and temperature may have a profound effect upon the development of marine invertebrates, but detailed studies in this area are surprisingly scarce even for the well-studied crustaceans. In recent years, a few papers have been published dealing with the larval development of some decapod crustaceans, particularly brachyurans, under different combinations of salinity and temperature. Costlow, Bookhout & Monroe (1960, 1962, 1966) examined the effect of salinity–temperature combinations on the larval development of *Sesarma cinereum*, *Panopeus herbstii*, and *Rhithropanopeus harrisii*, and Ong & Costlow (1970) studied the effects on *Menippe mercenaria*. More recently, Christiansen & Costlow (1975) looked at the effects of various salinities in combination with cyclic temperatures on the larval development of *Rhithropanopeus harrisii*. Sastry (1970) attempted to determine the salinity and temperature requirements for larval development and best survival of *Cancer irroratus* and *Panopeus herbstii*. Studies on non-brachyuran decapods are even less common but include Sandifer's (1975) work on the caridean grass shrimp *Palaemonetes vulgaris* and the study of Robertson (1968) on the macruran sand lobster *Scyllarus americanus*.

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There are no comparable studies published for anomuran decapod crustaceans. Coffin (1958) examined chlorinity and temperature separately during the development of *Pagurus samuelis*, Bookhout (1964) looked at the effects of different salinities on the development of *Pagurus bernhardus*, and Roberts (1971) made a similar study on *Pagurus longicarpus*. One purpose of this investigation is to provide the first published account of the combined effects of different salinities and temperatures on the larval development of an anomuran decapod, *Clibanarius vittatus* (Bosc), and the second will be to use these data to help explain the geographic distribution of this species.

Clibanarius vittatus is a very common estuarine hermit crab species in the western Atlantic, ranging from the Potomac River, Virginia, to Rio de Janeiro, Brazil (Williams, 1965). Lang & Young (1977) described the five zoeal stages and megalopa, complete with intermolt duration times and survival values for rearing conditions of 25 °C and 25‰ salinity, but did not provide data for other temperatures or salinities. The present investigation examines the larval development of *C. vittatus* in every combination of four salinities and five temperatures.

Materials and Methods

During the late spring and early summer of 1976, ovigerous females of *C. vittatus* were collected by hand from shallow water in the North Inlet estuary, Georgetown, South Carolina, U.S.A. (temperature, 23–27 °C; salinity, 20–23‰). Shells were carefully removed by cracking in a vise, and the shell-less females were put in individual 9 cm 'Carolina' culture dishes containing filtered natural sea water of 25‰ salinity and maintained at 25 °C under a 15L:9D light schedule. When several broods hatched simultaneously, larvae were combined into one pool; single hatches were not used in experiments to guard against individual variations among crabs. From the pool, the most active larvae, in groups of 90, were put in individual compartments of plastic boxes, each compartment measuring 53 × 62 × 39 mm deep and containing ≈ 20 ml filtered natural sea water of the appropriate salinity. Sea water of 25 and 30‰ salinity was obtained by collection; lower salinities were obtained by dilution with distilled water. Each rearing group was placed in an environmental chamber at the correct temperature and maintained on a 15L:9D light schedule. All combinations of four salinities (15, 20, 25, and 30‰) and five temperatures (15, 20, 25, 30, and 35 °C) were tested. Two drops of concentrated freshly hatched *Artemia* nauplii (San Francisco Bay) were added daily as food, and water was changed every three days. Preliminary investigations indicated no difference in survival or development if the water was changed daily, every other day, or every three days. Each larva was examined daily for evidence of molting or death. Individual records were kept for each of the 1800 larvae to determine accurate times for duration of larval stages and survival values for each experimental condition.

RESULTS

No development was observed in any salinity at 15 °C; all larvae remained in zoeal stage I until death. Early survival values were not significantly different from early survival at the other experimental temperatures, but by Day 20 the overall number of larvae surviving had dropped to 0 in 15‰, 6 in 20‰, 1 in 25‰, and 11 in 30‰ salinity. No larva survived longer than 35 days at 15 °C and only 18 out of 360 survived longer than 20 days.

Partial development was observed in all other experimental conditions. The degree of development was very similar for 20 and 35 °C. At 20 °C, 1 larva reached stage V in 15‰ and 1 stage IV in 20‰; 3 reached megalopa in 25‰, and 1 in 30‰. At 35 °C, 6 larvae survived to stage II in 15‰, and 5 to stage IV in 20‰; 1 reached megalopa in 25‰, and 2 in 30‰. Greater development and better survival were found at temperatures of 25 and 30 °C. Megalopa was the final stage reached in salinities of 15 and 20‰ (at 25 °C, 8 megalopae were obtained in 15‰ and 14 in 20‰; at 30 °C, 1 was obtained in 15‰ and 13 in 20‰). Complete development through metamorphosis to first juvenile crab occurred only in the remaining four test combinations. At 25 °C, 3 juvenile crabs were obtained in 25‰ and 2 in 30‰; at 30 °C, 15 were obtained in 25‰ and 3 in 30‰.

At any given temperature, either little difference was seen in development rates among the various salinities tested, or no trend was apparent if differences did exist (see Fig. 1; in all Figures, data for 15 °C are not included since there was no development). At any given salinity, however, temperature had a profound effect upon the rate of development (see Fig. 2). In all salinities, higher temperatures resulted in a considerable reduction in time required to reach each stage. For example, in 25‰ salinity, larvae reared at 30 °C reached zoeal stage V generally before larvae reared at 20 °C reached stage III. The most pronounced effect on development rate is seen in the temperature interval from 20 to 25 °C, with persistent but less striking effects at the intervals of 25 to 30 °C and 30 to 35 °C. With few exceptions, there is no difference in the overall period of time required for any zoeal stage to complete ecdysis to the succeeding stage at different temperatures; it is the duration of each instar between molts which is greatly reduced at higher temperatures (see Table I). For example, at 30‰ salinity, the mean duration of zoeal stage III was 13.4 days at 20 °C and only 7.1 days at 30 °C. The most rapid development took place at 30 °C and 25‰ salinity (to megalopa, 25 days minimum, 32.8 days average; to first crab, 44 days minimum, 53.8 days average).

Lang & Young (1977) noted a shortened developmental series of four zoeal stages prior to megalopa where a more advanced stage IV (IVb) zoea molts directly to megalopa, omitting V. This shortened series was observed a number of times in the present investigation in several combinations of salinity and temperature. It did not happen at 20 or 35 °C, but was seen 18 times at 25 °C (twice each in 15 and 20‰, and 7 times each in 25 and 30‰), and 53 times at 30 °C (8 times in 20‰, 31 times

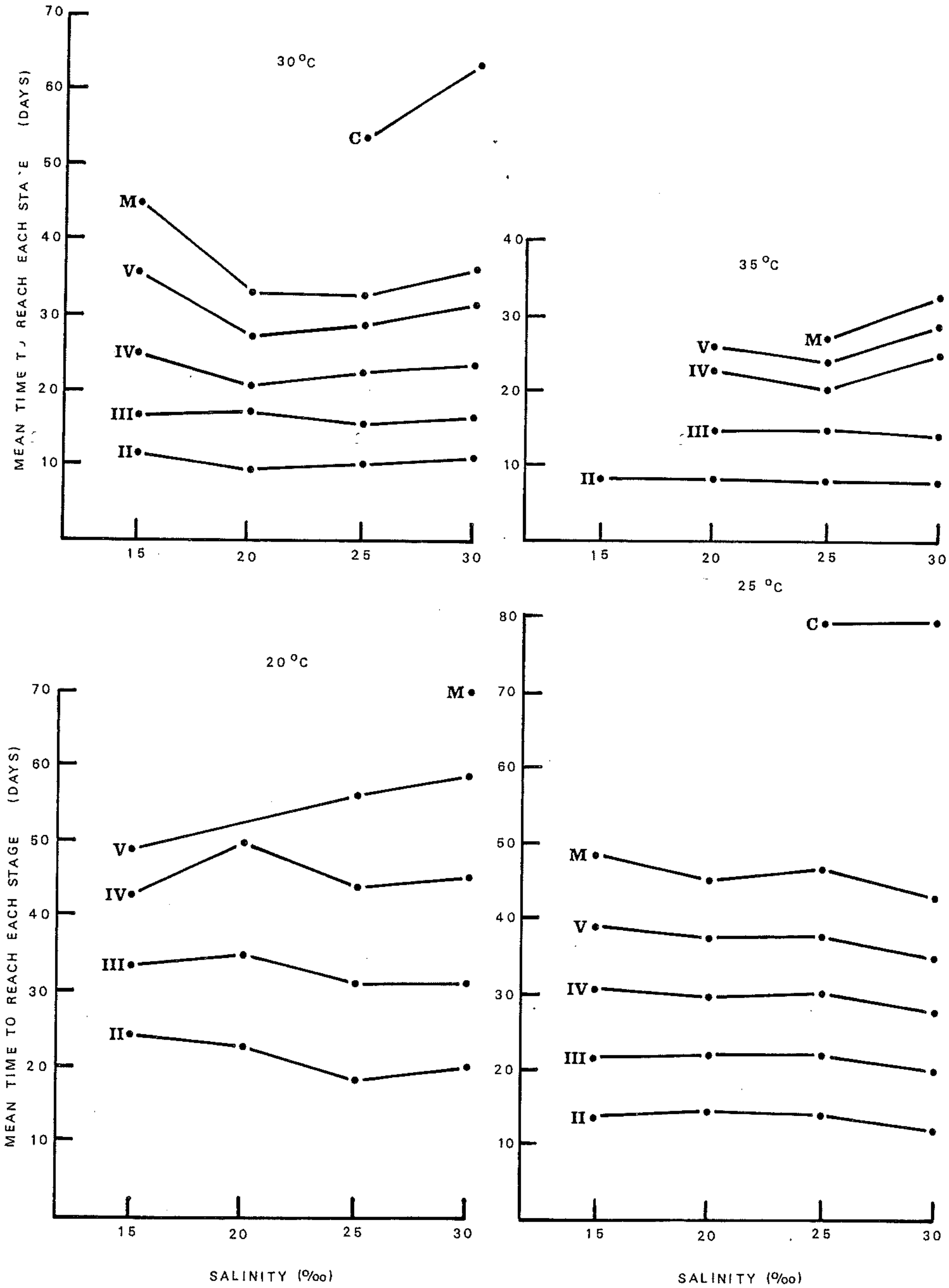

Fig. 1. Effect of salinity on the rate of larval development in *Clibanarius vittatus*: II–V, zoeal stages; M, megalopa; C, first juvenile crab; lines connecting each stage set are for ease in comparison and have no biological basis.

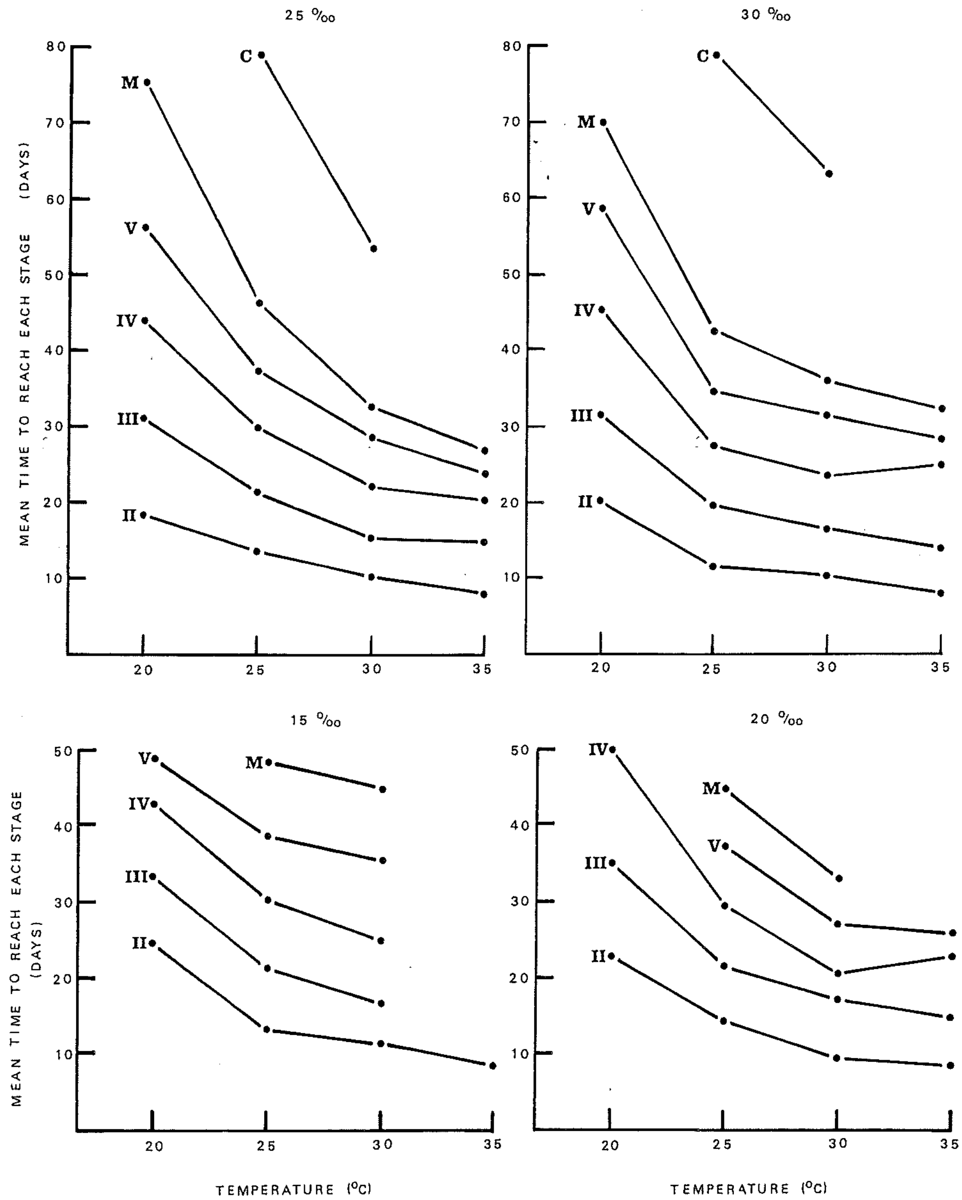

Fig. 2. Effect of temperature on the rate of larval development in *Clibanarius vittatus*: for explanation of symbols see Fig. 1.

TABLE I

Duration (in days) of the larval stages of *Clibanarius vittatus* reared under different combinations of salinity and temperature: duration is based on those larvae molting to the succeeding stage and does not include those individuals which died in stage.

Salinity (‰)	Temperature (°C)		Stage					
			Zoea I	Zoea II	Zoea III	Zoea IV	Zoea V	Megalopa
		Range	20–30	5–13	14	12	–	–
15	20	$\bar{x}$	24.4	7.7	14	12	–	–
		N	14	3	1	1	–	–
		Range	12–16	7–10	7–11	5–14	7–19	–
	25	$\bar{x}$	13.5	8.1	8.8	8.7	10.8	–
		N	34	26	25	19	6	–
		Range	8–16	2–9	4–15	8–13	8	–
	30	$\bar{x}$	11.7	5.2	8.3	11.0	8	–
		N	32	24	16	3	1	–
		Range	7–10	–	–	–	–	–
	35	$\bar{x}$	8.5	–	–	–	–	–
		N	6	–	–	–	–	–
		Range	19–27	16	15	–	–	–
20	20	$\bar{x}$	22.8	16	15	–	–	–
		N	5	1	1	–	–	–
		Range	10–20	5–15	6–12	6–12	8–11	–
	25	$\bar{x}$	14.3	7.7	8.6	8.3	8.6	–
		N	59	47	31	22	11	–
		Range	6–13	5–17	4–8	5–15	5–11	–
	30	$\bar{x}$	9.5	8.0	5.7	8.3	8.4	–
		N	27	19	11	13	5	–
		Range	6–13	3–10	2–15	–	–	–
	35	$\bar{x}$	8.6	6.4	7.3	–	–	–
		N	22	11	3	–	–	–
		Range	17–24	9–20	6–25	9–26	17–19	–
25	20	$\bar{x}$	18.5	12.6	13.1	13.2	17.7	–
		N	58	49	39	35	3	–
		Range	12–17	6–16	5–14	6–18	9–11	32–43
	25	$\bar{x}$	13.7	8.3	8.1	8.7	9.9	36.3
		N	46	35	40	40	29	3
		Range	6–17	2–11	4–16	2–13	5–15	14–30
	30	$\bar{x}$	10.3	5.4	7.2	9.3	9.8	19.9
		N	49	40	41	35	5	15
		Range	6–11	5	5–6	5	–	–
	35	$\bar{x}$	8.2	5	5.5	5	–	–
		N	12	1	2	2	–	–
		Range	16–24	6–15	9–25	10–24	14	–
30	20	$\bar{x}$	20.3	11.3	13.4	13.9	14	–
		N	70	49	34	16	1	–
		Range	10–15	7–14	5–12	5–16	8–11	30–49
	25	$\bar{x}$	11.6	8.2	8.0	7.2	9.2	39.5
		N	53	49	43	43	34	2
		Range	8–15	2–9	5–16	6–18	7–13	19–28
	30	$\bar{x}$	11.3	5.3	7.1	9.8	9.0	23.7
		N	43	37	29	25	9	3
		Range	6–11	5–10	10–16	5–6	5–6	–
	35	$\bar{x}$	8.0	6.7	13.0	5.5	5.5	–
		N	14	3	2	2	2	–

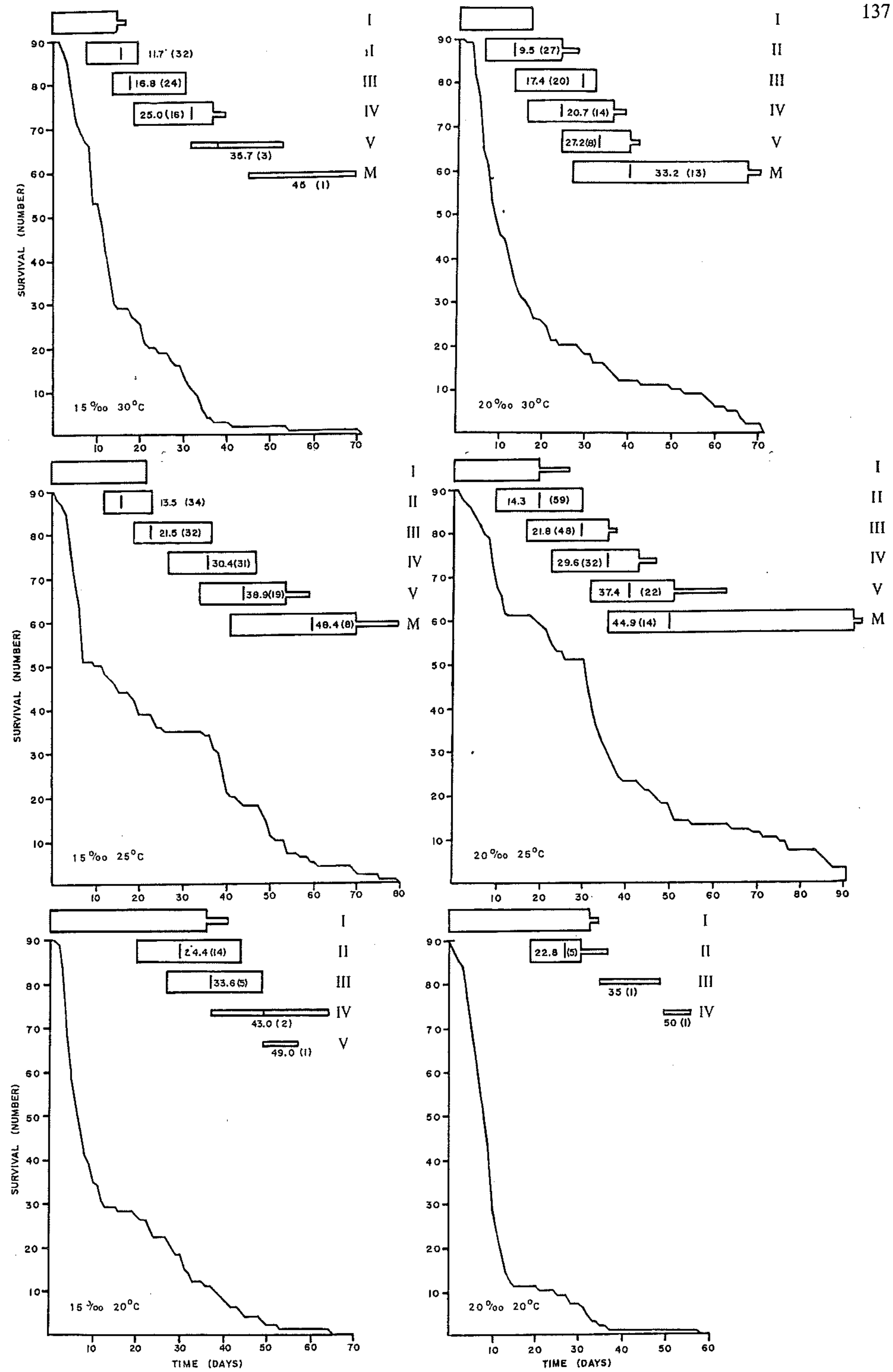

Fig. 3. Larval development of *Clibanarius vittatus* in salinities of 15 and 20‰: graph, number of larvae surviving against days after hatching; bars, zoeal stages I–V, megalopa (M), first juvenile crab (C); beginning of bar is first appearance of stage; end of bar is final disappearance of stage; dividing line within bar is last appearance of stage; number on bar is mean time to reach stage and (*N*); narrowed bars or sections of bars represent one or two individuals.

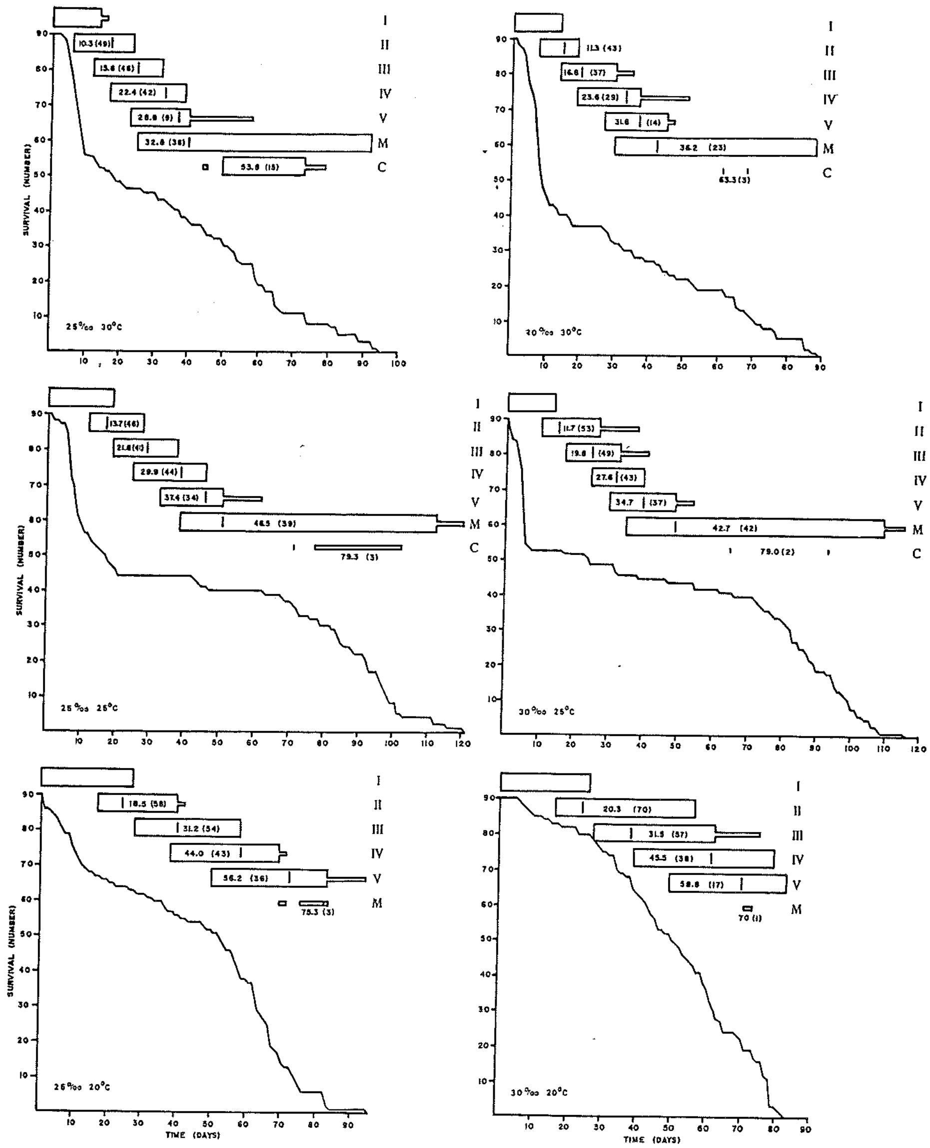

Fig. 4. Development of *Clibanarius vittatus* in salinities of 25 and 30‰; for explanation see Fig. 3.

in 25‰, and 14 times in 30‰). In fact, at 30 °C this short developmental series was the more common pathway to megalopa, comprising 89% of the stage IV zoeae and accounting for 82% of the megalopae obtained in 25‰, and 56% of the stage IV zoeae and 64% of the megalopae in 30‰. Of all the megalopae obtained at 30 °C, 70% molted directly from stage IVb larvae.

The overall survival of larvae, time after hatching until the first appearance of each developmental stage, time of last appearance of each stage, time of final disappearance of each stage, and mean time to reach each stage are shown in Figs 3 and 4. In general, mortality of zoeae was highest at the time of the first molt (from zoeal stage I to stage II) ranging from a low of 6% (20 °C, 20‰) to a high of 90% (20 °C, 30‰); all but two test conditions (20 °C, 25‰ and 20 °C, 30‰) had zoea I survival of less than 70% (see Table II). In most cases, survival of later stages improved

TABLE II

Larval stages of *Clibanarius vittatus* reared at different combinations of salinity and temperature: cumulative survival = survival from first zoeal stage to indicated stage (% of original, $N = 90$); within stage survival = survival within each stage (% of indicated stage to reach subsequent stage) in parentheses; * experiment terminated – no data for survival of juvenile crabs; zoea I omitted since within stage survival of zoea I = cumulative survival of zoea II.

Salinity (‰)	Temperature (°C)	Stage					
		Zoea II	Zoea III	Zoea IV	Zoea V	Megalopa	First crab
15	20	19 (36)	9 (25)	2 (50)	1 (0)	0 (–)	–
	25	46 (93)	42 (87)	36 (66)	21 (32)	9 (0)	0 (–)
	30	37 (76)	28 (68)	19 (18)	3 (33)	1 (0)	0 (–)
	35	7 (0)	0 (–)	–	–	–	–
20	20	6 (20)	1 (100)	1 (0)	0 (–)	–	–
	25	68 (85)	57 (65)	37 (70)	24 (54)	16 (0)	0 (–)
	30	31 (81)	23 (81)	17 (100)	9 (62)	16 (0)	0 (–)
	35	24 (64)	16 (36)	6 (0)	0 (–)	–	–
25	20	72 (92)	66 (75)	48 (79)	40 (80)	3 (0)	0 (–)
	25	59 (87)	49 (98)	49 (98)	40 (94)	43 (7)	3 (*)
	30	61 (87)	52 (100)	49 (89)	10 (67)	40 (39)	2 (*)
	35	4 (75)	3 (67)	2 (100)	1 (0)	1 (0)	0 (–)
30	20	90 (78)	70 (62)	43 (44)	19 (6)	1 (0)	0 (–)
	25	59 (94)	56 (90)	48 (100)	42 (92)	47 (5)	17 (*)
	30	48 (88)	42 (87)	36 (84)	16 (71)	26 (13)	3 (*)
	35	17 (27)	4 (75)	3 (100)	3 (67)	2 (0)	0 (–)

considerably, especially in combinations of higher salinities and temperatures. Overall survival to megalopa was greatest at 25 °C, 30‰ (47%) and to first crab at 30 °C, 25‰ (17%). Greatest mortality throughout the entire developmental process was near the end of the megalopa stage. In most experimental conditions, no megalopae metamorphosed successfully. In those conditions where some juvenile crabs were obtained, the megalopae success was 75% in 25 °C, 25‰; 5% in 25 °C, 20‰; 39% in 30 °C, 25‰; and 13% in 30 °C, 30‰.

DISCUSSION

Coffin (1958) reared the hermit crab *Pagurus samuelis* in some different chlorinities and temperatures, but did not discuss the results. From his tables, however, it can be seen that the duration of stages was somewhat longer at 17–18 °C than at 22–26 °C. No trend is evident relative to duration times at the different chlorinities tested, but mortality was lowest at a chlorinity of 16‰ (≈29‰ salinity), which was the only test condition in which megalopae were obtained. Bookhout (1964) found that between 15 and 35‰ salinity, the mortality of *Pagurus bernhardus* larvae increased with decreasing salinity and complete development to juvenile crabs occurred only at 30 and 35‰ salinity. Roberts (1971) found that *Pagurus longicarpus* would develop over a salinity range "at least from 18 to 30.5‰ and probably above".

In their work on *Sesarma cinereum*, Costlow, Bookhout & Monroe (1960) found that salinity was the more important environmental variable during development, especially relative to survival, whereas temperature affected the rate of development. Later studies (Costlow, Bookhout & Monroe, 1962, 1966; Ong & Costlow, 1970; Sastry, 1970; Christiansen & Costlow, 1975) suggested that salinity is not as important as temperature in affecting the larval development of brachyurans. Generally, increased temperature results in more rapid development and salinity has either slight effects or no trend. These findings are also confirmed in the work on non-brachyuran decapods by Robertson (1968) and Sandifer (1973). The data from the present study indicate that the same conclusions are valid for an anomuran. Survival and rate of development in *Clibanarius vittatus* were optimal at higher salinities and moderate temperatures with complete development to juvenile crabs only at combinations of 25 and 30‰ salinity with 25 and 30 °C.

Additional studies in this laboratory (Young, unpubl.) have shown that adult *C. vittatus* subjected to various temperatures and salinities have 90% survival after 48 h for temperatures ranging from 5 to 35 °C at optimal salinities (20–32‰). These tolerance limits exceed those shown by two other common hermit crab species tested, *Pagurus longicarpus* (12–20 °C in 25–30‰ salinity) and *P. pollicaris* (10–23 °C in 24–37‰ salinity). The low thermal tolerances of the *Pagurus* species are not as great as for *Clibanarius vittatus*, yet the geographical range along the western coast of the Atlantic extends northwards to Nova Scotia for *P. longicarpus* and to Massachusetts for *P. pollicaris*, while *C. vittatus* are not collected north of Virginia. The thermal tolerance of *C. vittatus* adults suggests that temperature is not the limiting factor in the geographical distribution of the species; the inability of the larvae to develop at low temperatures, however, may be responsible. *C. vittatus* larvae need ≈ two months at 25 °C and 1.5 months at 30 °C to metamorphose into juvenile crabs. The water north of Virginia is not warm enough for a sufficiently long period to allow complete development. Therefore, although adult *C. vittatus* could survive, the species is not found north of Virginia because of its inability to establish a breeding population. *Pagurus pollicaris*, on the other hand, has been reared at 16 °C (Nyblade, 1970) and *P. longicarpus* at 20 °C (Roberts, 1971), indicating that the northern geographic distribution of these species is not limited by the thermal requirements for larval development.

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