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Physiological Studies on *Cancer irroratus* Larvae. II. Effects of Temperature and Salinity on Physiological Performance*

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ABSTRACT: Larvae of the rock crab *Cancer irroratus* were cultured under specific environmental regimes to examine the influence of temperature and salinity on respiration and excretion rates during development. In addition, the type of biochemical substrate used for energy production was determined. The allometric relationship between oxygen consumption and body weight ($\dot{V}O_2$) was found to be affected by temperature but not by salinity. Larvae cultured at 10 °C exhibited a significantly ($P \leq 0.05$) higher regression coefficient (1.35) than did larvae maintained at either 15 ° (0.80) or 24 °C (0.87). In contrast, the relationship of ammonia excretion rate ($\dot{V}_{NH-N}$) to body weight were not affected by temperature but were affected by salinity. Larvae cultured in 15 °C at 25 ‰ S released significantly greater amounts of ammonia than did larvae of similar size at either 30 ‰ or 35 ‰ S. The atomic ratio of oxygen consumed to nitrogen excreted, the O : N ratio, fluctuated during development for larvae maintained in all environmental regimes tested. For larvae cultured at 10 °/30 ‰ S, the O : N ratio was low indicating that protein was the primary substrate used as an energy source. At the other conditions tested, the O : N ratio generally remained high indicating a combined use of protein, lipids and carbohydrates as energy sources. These data suggest that the successful development and recruitment of rock crab larvae is influenced by environmental conditions within their tolerance limits where physiological performance is impaired to such an extent that they are less fit to effectively compete within the zooplankton community.

INTRODUCTION

Laboratory studies have furthered our understanding of the effects of environmental conditions on the development of brachyuran larvae. Tolerance to temperature and salinity levels have been established for many species (Costlow and Bookhout, 1962; Costlow et al., 1966; see 'Marine Ecology': Kinne, 1970, 1971, 1977 for reviews). Within the zone of tolerance, environmental conditions have been shown to affect development rate, intermolt duration and growth (Costlow and Bookhout, 1971; Christiansen and Costlow, 1975; Johns, 1981).

In addition, studies have assessed the impact of environmental variation on the function of physiological and biochemical systems in crab larvae. Some work has been reported for respiratory rates and metabolic-temperature responses of several species under contrasting conditions (Vernberg and Costlow, 1966; Belman and Childress, 1973; Sastry and McCarthy, 1973; Schatzlein and Costlow, 1978; Sastry, 1979), as have works detailing the osmoregulatory ability of developing larvae (Kalber and Costlow, 1966; Kalber and Costlow, 1968; Foskett, 1977). Biochemical changes brought about by differing environmental conditions have also been considered (Frank et al., 1975; Sulkin et al., 1975; Tucker, 1977; Morgan et al., 1978; Sastry and Ellington, 1978).

Although the above studies give an appreciation for the range of responses of individual physiological and biochemical systems to environmental variations, no

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single study has considered the integration of these systems into a whole organism response. Such a holistic approach is needed to adequately interpret the effects of the environment on larval development since organisms seldom respond to environmental change through adjustments in an individual physiological or biochemical rate but rather respond as an integrated whole.

A series of experiments were designed to study the effects of specific environmental regimes on larval development of the rock crab *Cancer irroratus*. The effects of temperature and salinity on survival, development rate, intermolt duration and larval size were determined (Johns, 1981). Further, an investigation was made of the energy balance of the larvae at various environmental conditions to determine how temperature and salinity affect energy flow (Johns, 1980). The present paper examines the influence of temperature and salinity on the physiological response of rock crab larvae and the degree to which respiration and excretion rates are integrated during development. In addition, the type of biochemical substrate used for energy production was determined.

MATERIALS AND METHODS

Gravid *Cancer irroratus* were collected by otter trawl from the West Passage of Narragansett Bay, Rhode Island, from December to May of 1977 through 1979. Methods for laboratory maintenance of the gravid adults, procurement of newly-hatched zoeae and the mass culture techniques used in this study are presented in detail elsewhere (Johns, 1981).

Respiration rates, excretion rates and the calculation of an O : N ratio were made for larvae cultured in the following temperature-salinity conditions: 10 °C/30 ‰ S, 15 °C/25 ‰ S, 15 °C/30 ‰ S, 15 °C/35 ‰ S, 24 °C/30 ‰ S. Respiration rates and excretion rates were determined for all 5 zoeal stages. These determinations were made between 24 and 36 h after hatching (for Stage I zoeae). For all other stages, the larvae used in the experiments were between 24 and 36 h following the molt from the previous larval stages. Mean molting times for each larval stage were taken from a concurrent study (Johns, 1981). All larvae were fed daily with newly-hatched Brazilian-strain *Artemia* (Johns et al., 1980).

Routine oxygen consumption rates were determined with all glass micro-respirometers (Grunbaum et al., 1955) having a 0.3 mm capillary bore. The number of zoeae used in each determination varied with larval size and ranged from 6 larvae for Stage I zoea to 1 larva for Stage V zoea. The respirometers were allowed to equilibriate for at least 30 min prior to the initial readings; readings were taken every 15 min for 3 h. To avoid interference from possible circadian patterns of respiration, all determinations were made between 1200 and 1800 h. Following the respiration run, the crab larvae were rinsed in 0.9 % ammonium formate (W : V), dried in a 60 °C oven for 24 h and weighed on a Perkin-Elmer Autobalance* to the nearest 1.0 µg.

Excretion rates were determined for groups of larvae that were placed in 10 ml of 0.45 µm filtered seawater for up to 5 h. As in the respiratory rate determinations, the number of zoeae used for each determination depended on larval size. For zoeal Stages I and II, 10 larvae were used, with 8 larvae being utilized for Stage III and 5 larvae for both Stages IV and V. Ammonia concentrations were determined after Solorzano (1969). In addition, atomic O : N ratios were calculated for each larval stage to determine types of biochemical substrate used for energy production (Corner and Cowey, 1968).

The allometric relationship of both respiratory rate and excretory rate to body size was fitted to the following equation:

$$M = aW^b \quad (1)$$

where M = oxygen consumption rate or ammonia excretion rate; W = weight; a and b = constants. Using log-transformed data, the above mathematical relationship was fitted to a linear model using least square linear regression analysis. Subsequent statistical tests (analysis of covariance) could then be used to determine the effects of rearing conditions on these size-dependent relationships (Snedecor and Cochran, 1967).

The influence of larval development on weight-specific respiration rates was determined using one-way analysis of variance. If significant differences (at $P = 0.05$) were found among the treatments at each zoeal stage, a Duncan's Multiple Range test was used to determine where the differences occurred (Snedecor and Cochran, 1967).

RESULTS

The relationship between oxygen consumption ($\dot{V}_{O_2}$) and body size for *Cancer irroratus* larvae maintained at various temperatures in 30 ‰ S was linear (Fig. 1). At all temperatures tested, $\dot{V}_{O_2}$ increased with increasing body weight. The regression coefficient (b, a measure of the proportionality of respiratory rate to body weight), for larvae cultured at 15 °C ($b = 0.80$) and 24 °C ($b = 0.87$), was not significantly different from

* Mention of trade names does not imply endorsement by the United States Environmental Protection Agency

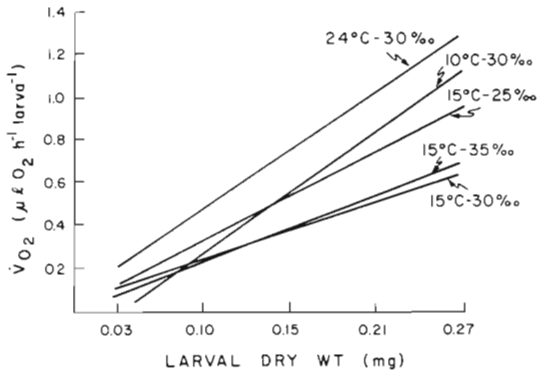

Fig. 1 *Cancer irroratus*. Respiration rates ($\dot{V}_{O_2}$) of larvae cultured in various combinations of temperature and salinity. Plotted lines represent log transformed data fitted to the linear regression model: log $\dot{V}_{O_2}$ = log a + b log dry wt. Use Table 1 for numerical parameters and correlation coefficients of these lines

Table 1. *Cancer irroratus*. Parameters of fitted regression lines, and their correlation expressing the allometric relationship of $\dot{V}_{O_2}$ and body weight for zoeal stages cultured at various conditions of temperature and salinity. (n): number of respiration rate determinations; b: regression coefficient for the fitted regression line; r: correlation coefficient

Culture condition (°C) – (‰ S)	(n)	b	r	Regression line
10–30	30	1.35	.91	$\log \dot{V}_{O_2} = -0.62 + 1.35 \log$ dry wt.
15–25	30	0.97	.90	$\log \dot{V}_{O_2} = -0.56 + 0.97 \log$ dry wt.
15–30	90	0.80	78	$\log \dot{V}_{O_2} = -0.35 + 0.80 \log$ dry wt.
15–35	30	0.93	.95	$\log \dot{V}_{O_2} = -0.40 + 0.93 \log$ dry wt.
24–30	30	0.87	.90	$\log \dot{V}_{O_2} = -0.69 + 0.87 \log$ dry wt.

each other; however, both of these values were significantly lower than the regression coefficient for larvae reared at 10 °C (b = 1.35; Table 1).

Salinity did not appear to influence the $\dot{V}_{O_2}$ to body size relationship (Fig. 1). Regression lines for larvae cultured in 3 salinities (25, 30, and 35 ‰ S) at 15 °C

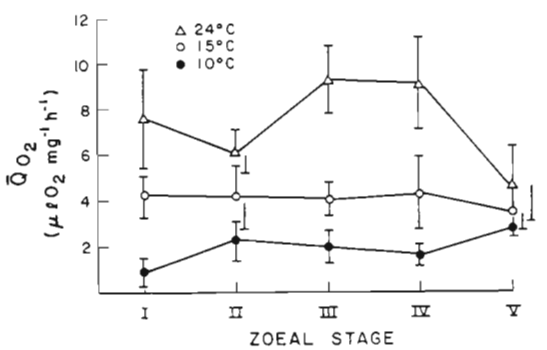

Fig. 2. *Cancer irroratus*. Weight-specific respiration rates (mean ± 1 std. deviation) for larvae cultured at various temperatures in 30 ‰ S

Table 2. *Cancer irroratus*. Analysis of variance of interstage weight-specific respiration rates for zoeal stages cultured at various conditions of temperature and salinity

Culture condition (°C) – (‰ S)		S.S.	d.f.	M.S.	F-Ratio
10–30					
	Treatment	10.97	4	2.74	–
	Error	7.64	19	0.40	–
	Total	18.61	23	–	6.82*
15–35					
	Treatment	7.01	4	1.75	–
	Error	68.23	55	1.24	–
	Mean	75.24	59	–	1.41
15–30					
	Treatment	5.11	4	1.28	–
	Error	95.66	68	1.41	–
	Total	100.68	72	–	0.91
15–35					
	Treatment	8.33	4	2.08	–
	Error	38.86	22	1.68	–
	Total	45.19	26	–	1.24
24–30					
	Treatment	81.38	4	20.35	–
	Error	85.66	21	4.08	–
	Total	167.04	25	–	4.99*

* F-ratio values are significant ($P \leq 0.05$)

were not significantly different (F = 1.58, d. f. 2,34) according to an analysis of equality of the three regression lines. Although regression coeffients for these lines ranges from 0.80 to 0.97, the variaton in $\dot{V}_{O_2}$ at each salinity did not allow for statistical differentiation (P = 0.05) of the data (Table 1).

Interstage weight-specific respiration rates ($\bar{Q}_{O_2}$) for larvae cultured in 30 ‰ S, but at different temperatures, varied to some degree (Fig. 2). At 15 °C, $\bar{Q}_{O_2}$ rates were similar in all 5 zoeal stages. At the other 2 temperatures tested, however some differences in weight-specific respiration were found (Table 2). Despite the stage-to-stage differences seen in larvae cultured at 10 ° and 24 °C, the basic pattern of con-

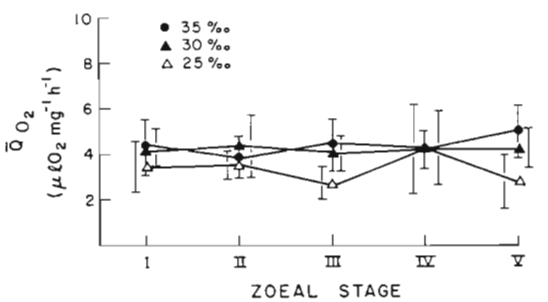

Fig. 3. *Cancer irroratus*. Weight-specific respiration rates (mean ± 1 std. deviation) for larvae cultured in various salinities at 15 °C

stancy in $\bar{Q}_{O_2}$ during zoeal development exhibited by larvae maintained at 15 °C is still apparent.

Salinity had very little effect on weight-specific respiration rates for larvae maintained at 15 °C (Fig. 3). No significant differences were found in $\bar{Q}_{O_2}$ between any of the zoeal stages of rock crab larvae cultured in 25, 30, and 35 ‰ S (Table 2).

The allometric relationship of excretion rate ($\dot{V}_{NH_4-N}$) to body weight for larvae maintained under various temperature-salinity conditions is presented in Fig. 4.

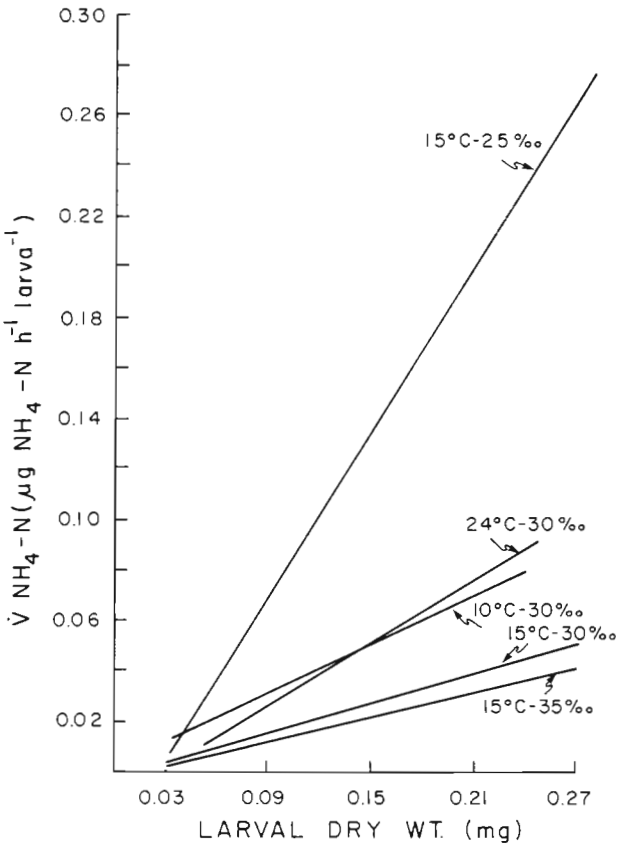

Fig. 4. *Cancer irroratus*. Excretion rates ($\dot{V}_{NH_4-N}$) of larvae cultured at various combinations of temperature and salinity. Plotted lines represent log transformed data fitted to the linear regression model: log $\dot{V}_{NH_4-N}$ = log a + b log dry wt.; use Table 3 for numerical parameters and correlation coefficients of these lines

In all cases, the regression coefficient, *b*, for the fitted regression lines was greater than 1.00 (Table 3). Temperature appears to have a constant effect on this allometric relationship since the regression coefficients for larvae culture at 10 °, 15 ° and 24 °C were not significantly different from each other. The intercept of the 3 regression lines were different, however, indicating that larvae cultured at 10 ° and 24 °C liberate significantly lower amounts of ammonia than larvae of a similar size maintained at 15 °C.

Table 3. *Cancer irroratus*. Parameters of fitted regression lines, and their correlation coefficients expressing the allometric relationship of $\dot{V}_{NH_4-N}$ and body weight for zoeal stages cultured at various conditions of temperature and salinity. (n): number of excretion rate determinations; *b*: regressions coefficient for the fitted regression line; *r*: correlation coefficient

Culture condition (°C) – (‰ S)	(n)	*b*	*r*	Regression line
10–30	23	1.15	.94	$\log \dot{V}_{NH_4-N} = -0.34 + 1.15 \log$ dry wt.
15–25	24	1.54	.91	$\log \dot{V}_{NH_4-N} = +0.21 + 1.54 \log$ dry wt.
15–30	35	1.12	.91	$\log \dot{V}_{NH_4-N} = -0.59 + 1.12 \log$ dry wt.
15–35	22	1.15	.94	$\log \dot{V}_{NH_4-N} = -0.65 + 1.15 \log$ dry wt.
24–30	27	1.25	.84	$\log \dot{V}_{NH_4-N} = -0.24 + 1.25 \log$ dry wt.

Salinity affected the amount of ammonia liberated by the rock crab larvae with $\dot{V}_{NH_4-N}$ increasing as size increased and salinity decreasted (Table 3). Larvae cultured at 25 ‰ S (b = 1.54) released significantly ($P \leq 0.05$) greater amounts of ammonia than did larvae of a similar size at either 30 ‰ S (b = 1.12) or 35 ‰ S (b = 1.15).

The atomic ratio of oxygen consumed to nitrogen excreted, the O:N ratio, fluctuated during larval development for larvae reared in all conditions tested

Table 4. *Cancer irroratus*. Calculated O:N ratios for zoeal stages cultured at various conditions of temperature and salinity

Zoeal stage	Culture condition (°C) – (‰ S) 10–30	15–25	15–30	15–35	24–30
I	5.86	18.14	28.56	25.36	17.56
II	4.40	15.33	32.09	31.88	37.00
III	7.00	4.95	12.90	12.65	47.18
IV	8.56	13.40	12.20	9.76	25.15
V	12.40	12.44	19.05	15.44	6.93

(Table 4). Oxygen-to-nitrogen ratios of approximately 7 indicate that protein is the sole substrate used for energy production, while increasing values are interpreted as an increasing reliance on carbohydrates and/or lipids. For rock crab larvae cultured at 10 °C/30 ‰ S, the O:N ratio was particularly low throughout development, suggesting that protein was the primary substrate used as an energy source. At the other culture conditions tested, the O:N ratios remained above 7 during most of the developmental period, except in two instances (Stage III at 15 °C/25 ‰ S and Stage V at 24 °C/30 ‰ S).

DISCUSSION

The relationship of $\dot{V}_{O_2}$ to larval body size is one physiological parameter altered by culture conditions. Typically, this regression coefficient for most crustaceans falls between 0.67 and 1.00 (Wolvekamp and Waterman, 1960) and this has generally been the case for crustacean larvae (Mootz and Epifanio, 1974; Logan and Epifanio, 1978; Schatzlein and Costlow, 1978; Johns and Pechenik, 1980). The same has been seen here in larvae maintained in salinities of 25, 30 and 35 ‰ S at 15 °C, and those maintained at 24 °C/30 ‰ S exhibited regression coefficients of between 0.80 and 0.97. However, larvae cultured at 10 °C/30 ‰ S had a regression coefficient of 1.34. This is unusual and represents a significant deviation for this relationship. A high regression coefficient was also reported by Capuzzo and Lancaster (1979) for lobster *(Homarus americanus)* larvae. These authors did not advance an explanation for their high *b* value (1.24), a value which is considerably higher than that (0.66) found by Logan and Epifanio (1978) for lobster larvae cultured under similar conditions. One possible cause for the deviation in the basic relationship between body weight and oxygen consumption in any species may be that the organisms were under physiological stress; this can be induced by culture conditions. For rock crab larvae, 10 °C represents a limiting temperature with only 20 % of the larvae maintained at this temperature completing larval development to the megalopa stage (Johns, 1981). Since metabolic responses of marine organisms change at the thermal limits (Vernberg and Vernberg, 1970) it might be expected that the allometric relationship would also be affected.

Weight-specific respiration rates ($\overline{Q}_{O_2}$) of larvae cultured at 10 °C/30 ‰ S and at 25, 30, and 35 ‰ S at 15 °C tended to change little during development, indicating little stage-specific sensitivity to temperature and/or salinity. Larvae cultured in 24 °C/30 ‰ S exhibited some slight temperature sensitivity during late zoeal development. Sensitivity of rock crab larvae to thermal conditions have also been reported by Sastry and McCarthy (1973) on studies detailing larval metabolic-temperature responses. Larvae were found to be more stenothermal during late larval development than during the earlier stages of development.

At a particular set of environmental conditions, weight-specific respiration rates typically decrease as an organisms grows and develops due to a disproportionate increase in tissue of low metabolic rate (Prosser, 1973). However, comparable literature values for other crustacean larvae indicate that this pattern may not follow for all crustacean larvae. Larvae of the stone crab *Menippe mercenaria* (Mootz and Epifanio, 1974) follow the expected pattern, as do larvae of *Emerita talpoida* (Schatzlein and Costlow, 1978; their Fig. 3). On the other hand, Capuzzo and Lancaster (1979) reported that weight-specific respiration rates for lobster larvae increase rather than decrease during larval development. Both the data of Sastry (1979) and that in this study, however, indicate that the weight-specific respiratory rate of *Cancer irroratus* larvae does not change during larval development. The same was also seen in *Libinia emarginata* larvae (Schatzlein and Costlow; 1979, their Fig. 3).

The reason for the above contrasting observations are not known. In developing crustacean larvae, there may not necessarily be an increase in low metabolic tissue, if major morphological and physiological changes do not occur during zoeal development such as suggested by Costlow (1968). The general tissue types present in late stage larvae and their metabolic activity may be similar to those present in larvae at hatching. Hence, metabolic demands per unit weight would remain unchanged. In support of this argument, Sulkin et al. (1975) found that larval development of the brachyuran *Rhithropanopeus harrisii* was due primarily to an increase in cell size rather than due to an increase in cell number or cell types. Also, the sequence of changes in different organ systems in the naupliar stages of the barnacle *Balanus balanoides* has been reported as slight with no major changes occurring until metamorphosis to the cyprid stage (Walley, unpubl. in Costlow, 1968).

In contrast to the amount of data available on the effects of body size and environmental conditions on respiratory rates, little is known concerning body size excretory rate relationships. Bayne and Scullard (1977) working with the adult mussel *Mytilus edulis* and Capuzzo and Lancaster (1979) working with *Homarus americanus* larvae have reported regression coefficients in excess of 1.00 for the allometric relationship of excretion rates to body size. This is in agreement with those values reported here for rock crab larvae. In addition, Bayne and Scullard (1977) found that the allometric relationship between excretion rates and size in the mussel was temperature independent, a fact also seen in the present study. Rates of ammonia production for a given size class of crab larvae, however, was significantly greater in larvae maintained at 24 °C/30 ‰ S and 10 °C/30 ‰ S than for larvae cultured at 15 °C/30 ‰ S. Salinity on the other hand affected both the excretion rate weight relationship as well as the amount of ammonia liberated per unit weight. Excretion rates increased as salinity decreased, with rates at 25 ‰ S substantially greater than that in larvae maintained at 30 and 35 ‰ S. Similar results have been reported for a variety of species and appears to be associated with the osmoregulatory mechanism (Emerson, 1969; Pandian, 1975; Kinne, 1976).

Although instances where physiological rates exceeded the normal range are indicative of physiological stress (Sastry and Miller, 1981), the measurement of individual physiological rate functions does not necessarily provide direct evidence that an organism's potential for survival will be reduced (Brett, 1958; Bayne, 1975). A better assessment of stress can be made by determining the atomic ratio of oxygen consumed to nitrogen excreted (Bayne, 1973; 1975; Widdows, 1978). An O:N ratio not only provides a measure of the integration of physiological functions but also gives some indication of the catabolic balance between the energy substrates. Low O:N ratios are indicative of protein being used as a source of energy; conversely, the higher the O:N ratio, the more carbohydrates and/or lipids are being utilized (Corner and Cowey, 1968). Since the biochemical composition of crustacean larvae is mostly protein (Frank et al., 1975; Capuzzo and Logan, 1979), utilization of dietary and body protein as an energy source diverts this resource from growth to maintenance needs.

Mobilization of protein to meet metabolic demands presents several problems regarding completion of larval development. The ability of larvae to survive in a particular set of environmental conditions is dictated by its ability to compete with other organisms with similar tolerance limits (Newell and Branch, 1980), where rapid growth is typically the key to predator avoidance and food procurement. In the present study, rock crab larvae appeared to rely on more than one biochemical substrate for energy production, with the use of protein being dependent on the culture condition. Larvae maintained at 10 °C/30 ‰ S were primarily using protein as an energy source while larvae in the other conditions appeared to be mobilizing carbohydrates and lipids to a greater extent in order to meet energy needs.

Larvae exhibit a capacity to compensate in most circumstances (15 ° to 24 °C in 30 to 35 ‰ S), with their physiological rate functions being well within the range expected for brachyuran larvae. Under some conditions (10 °C/30 ‰ S; 15 °C/25 ‰ S) however, there was a deviation from the norm for the physiological rates and a shift to protein as a source of energy. The physiological stress imposed on *Cancer irroratus* larvae in these conditions has the effect of reducing growth rates and increasing pelagic development times (Johns, 1981). This may represent a large loss of larvae prior to recruitment to the adult population since stressed larvae may be unable to compete for available food resources and avoid planktonic predators (Thorson, 1950; Vance, 1973; Strathmann, 1977). Hence, the successful development and recruitment of rock crab larvae to the benthos appears to be not only restricted by their tolerance limits, but is also influenced by conditions within these limits where physiological performance is impaired to such an extent that they are less fit to effectively compete within the zooplankton community.

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