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Ontogeny of Respiratory and Growth Responses of Larval Mud Crabs *Rhithropanopeus harrisii* Exposed to Different Temperatures, Salinities and Naphthalene Concentrations

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ABSTRACT: Larval mud crabs *Rhithropanopeus harrisii* were exposed continuously from hatching through the first crab stage to sublethal concentrations of naphthalene (0, 75, 150 or 300 $\mu g\ l^{-1}$) at several combinations of salinity and temperature (5, 15, or 25 ‰ S and 20°, 25° or 30 °C, respectively), the experimental design consisting of a complete 3 × 3 × 4 factorial. Respiration rates were determined under all treatment combinations for the second and fourth zoeal stage, megalops and first crab. In addition to a steady-state respiratory response at the rearing salinity, an osmotic-shock respiratory response was also assessed by respirometry immediately after transfer of larvae reared at 15 ‰ S to either 5 ‰ S (hypoosmotic shock) or 25 ‰ S (hyperosmotic shock). Respiratory rates increased with temperature for all stages. Increases in the respiration rate of larval stages, especially zoeal stages, also occurred in low salinities. The increase due to low environmental salinity was least in the juvenile crab, probably because of development of the osmoregulatory capacity of the gill. Respiratory response to hypoosmotic shock was similar to that observed for steady-state trials at 5 ‰ S, but the pattern for hyperosmotic shock was intermediate between the steady-state response at 15 and 25 ‰ S. Naphthalene exposure usually resulted in an increase in respiration rate (of the zoeal stages) in low salinities. However, when physical conditions were not stressful, naphthalene effects generally were not obvious. The weight of megalops from the various trials (environmental factor combinations) was determined as an indication of growth during zoeal develoment. Growth was affected substantially by higher temperatures and lower salinities, and slightly by lower salinity-higher naphthalene combinations. At optimal salinity, 15 ‰ S, no consistent effect of naphthalene on growth was apparent. This partial energy budget approach is seen as a useful method for assessing long-term sublethal stress in marine invertebrates.

INTRODUCTION

Comparative toxicity studies have shown that various crude and refined petroleum products differ in their toxicity to representative species of marine organisms (Anderson et al., 1974; Rice et al., 1977; Craddock, 1977; Johnson, 1977; Varanasi and Malins, 1977). Most investigators agree that low molecular weight aromatics contribute most, proportionally, to the toxicity of crude and refined oils (Moore and Dwyer, 1974). Mono- and diaromatics, in particular, were cited by Boylan and Tripp (1971). Anderson et al. (1974) considered naphthalene and alkylated naphthalenes to be particularly toxic components of a No. 2 fuel oil and bunker C residual oil.

Acute responses to spilled oil rarely are observed in nature except immediately after major oil spills. Since chronic low levels of petroleum hydrocarbons may predominate in near-shore environments, sublethal responses are the most biologically significant aspects of most organisms' response to such pollutants.

In this paper, we report the respiration rate and growth of selected larval stages of the mud crab, *Rhithropanopeus harrisii*, exposed throughout larval development to naphthalene at different combinations

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of levels of temperature and salinity. Bioassay information for the response of *R. harrisii* to this hydrocarbon has been given elsewhere (Laughlin and Neff, 1979a). Our purpose is to describe the sublethal responses of the larvae and to explain how homeostatic mechanisms are affected during exposure to a representative diaromatic hydrocarbon.

MATERIALS AND METHODS

Ovigerous mud crabs *Rhithropanopeus harrisii* were collected in the Indian River, Florida (USA) near the mouth of the Sebastian River and transported by air to Texas. In the laboratory, they were segregated individually into 18-cm finger bowls containing 400 ml of sea water (Instant Ocean, Aquarium Systems, Eastlake, Ohio). Temperature and salinity during the prehatch incubation period were 23° ± 2 °C and 15 ‰ S, respectively. Freshly hatched *Artemia* nauplii were provided as food for the adults and for the larvae when they hatched.

The larvae were reared in factorial combinations of three temperatures, three salinities and four hydrocarbon concentrations (treatments). Temperatures and salinities were, respectively 20°, 25°, or 30 °C and 5, 15 or 25 ‰ S. Naphthalene, obtained from Chemical Samples Co. (Columbus, Ohio), was dissolved in acetone (Nanograde®, Mallinckrodt) and appropriate microliter quantities were added to sea water of each salinity to give nominal exposure concentrations of 0 µg l^{-1}, (100 µl acetone l^{-1} sea water), 75, 150 or 300 µg l^{-1} naphthalene.

Nominal exposure concentrations will be used when referring to naphthalene concentrations in the water. However, because naphthalene is volatile, and loss rate to the air follows first order loss kinetics, actual exposure concentrations were lower than indicated by initial (i.e. nominal) values. Both temperature and salinity would affect this time-dependent loss, temperature being most important. In a study of the loss of naphthalene from the water-soluble fraction of No. 2 fuel oil (Laughlin et al., 1979a), we found that the half time for naphthalene retention would be less than 12 h at 30 °C and 3–4 d at 20 °C. These numbers would probably be close to the parameters of time-dependent naphthalene loss from the present experimental system. While such volatility-induced losses might be suspected of skewing sublethal toxicity patterns, the data will show that animals exposed to naphthalene under physical factor regimes maximizing hydrocarbon loss were the most severely affected.

Before initiation of naphthalene exposure, larvae were acclimated as detailed elsewhere (Laughlin and Neff, 1979a) to the temperature-salinity test conditions used if those conditions differed from the 15 ‰ S, 23 °C one used for hatching. Graded salinity changes lasting 18 h were used for salinity acclimation. The major difference between the procedures in the earlier experiment and this one is that the larvae were reared in mass culture in 18-cm finger bowls containing 400 ml of seawater-naphthalene exposure medium.

It would have been impossible to test all stages and treatment combinations from a limited number of hatches. Therefore, specific stages were selected. These were the second zoea, fourth zoea, megalops and first crab stages.

Respiration rates were determined by oxygen electrode (Radiometer Blood Gas Analyzer), using all-glass syringes as respirometry chambers as described elsewhere (Laughlin et al., 1979b). Respiration rates were determined for 2 individuals in 2–3 ml of seawater during a 2-h respirometry period. The choice of respirometer volume was appropriate to maintain dissolved oxygen concentrations greater than one-half of air saturation during the 2-h test period. The protocol required the use of 6 replicates for each factor combination, but occasionally this was reduced because of a shortage of animals, particularly for later stages.

After respirometry, the animals were removed from the syringes, rinsed briefly in distilled water and dried at 60 °C for at least 48 h. Dry weights were determined to the nearest 0.1 µg on a Mettler M-7 microbalance.

Respiration rates are expressed as ml O_2 g dry wt^{-1} h^{-1}. The data were analyzed statistically using the General Linear Models (GLM) Procedure of SAS '76 (Barr et al., 1976). This statistical program performs a multiple regression analysis of variance and provides estimates of the coefficients for the regression equation used to model the data. Specifically, the regression model tested here was second order with respect to temperature, salinity and naphthalene. The model was first order with respect to the acclimation condition since the experimentals were in either of two states during testing, acclimated or 'shocked'. These were plotted using the Procedure Scatter of SAS '76 to produce response surface diagrams.

RESULTS

The single most important factor affecting respiration rate of larval *Rhithropanopeus harrisii* was their size. In a previous paper, we showed that the weight-specific respiration rate of these stages was proportional to the weight raised to the exponent −0.36 (Laughlin and Neff, 1980). In this paper we present the respiration rate data by stage to demonstrate more clearly the effects of physical factors and naphthalene exposure.

Second Zoeae

In general, there was a strong effect of salinity on the steady-state respiratory response, respiration rates tending to decrease with increasing salinity over the range tested. Also evident was a marked interaction of the two factors during this stage of development (Table 1).

The mean respiratory rate for control animals acclimated to 5 ‰ S and tested at this salinity decreased with increasing temperature (Fig. 1). At 20 °C, all naphthalene-exposed animals had mean respiratory rates below control values, but the situation was reversed at the two higher temperatures. At 20 °C, the highest naphthalene exposure groups displayed the largest decrease in respiration rates compared to control values; at 25 °C, this group had the greatest increase over control values. At 5 ‰ S, a temperature

Table 1. *Rhithropanopeus harrisii*. Synopsis of multiple regression analyses of the effects of temperature (T), salinity (S) and naphthalene (N) exposure on respiration rates of selected larval stages. C- condition of either steady state with test salinity or osmotic shock during respirometry

Stage	Correlation coefficient	Significance of F statistic		
		$\geq .05$	$.05 > F > .01$	$\leq .01$
Second zoeae	0.78	S, C, S^2, N^2, T × S, T × C	N × C	T, N, T^2, T × N, S × N, S × C, T × S × N, T × S × C
Fourth zoeae	0.83	N, S^2, N^2, T × N, S × N, N × C, S × C, T × S × N, T × S × C	C, T × S	T, S, T^2, T × C
Megalops	0.70	N N^2 C, T × S, T × N, S × N, T × C, N × C, T × N × C	S	T, T^2, S^2, S × C, T × S × C
First crab	0.80	S, C, S^2, N^2, T × S, S × N, T × C, N × C, S × C, T × S × C	N, T, T × S × N	T^2, T × N

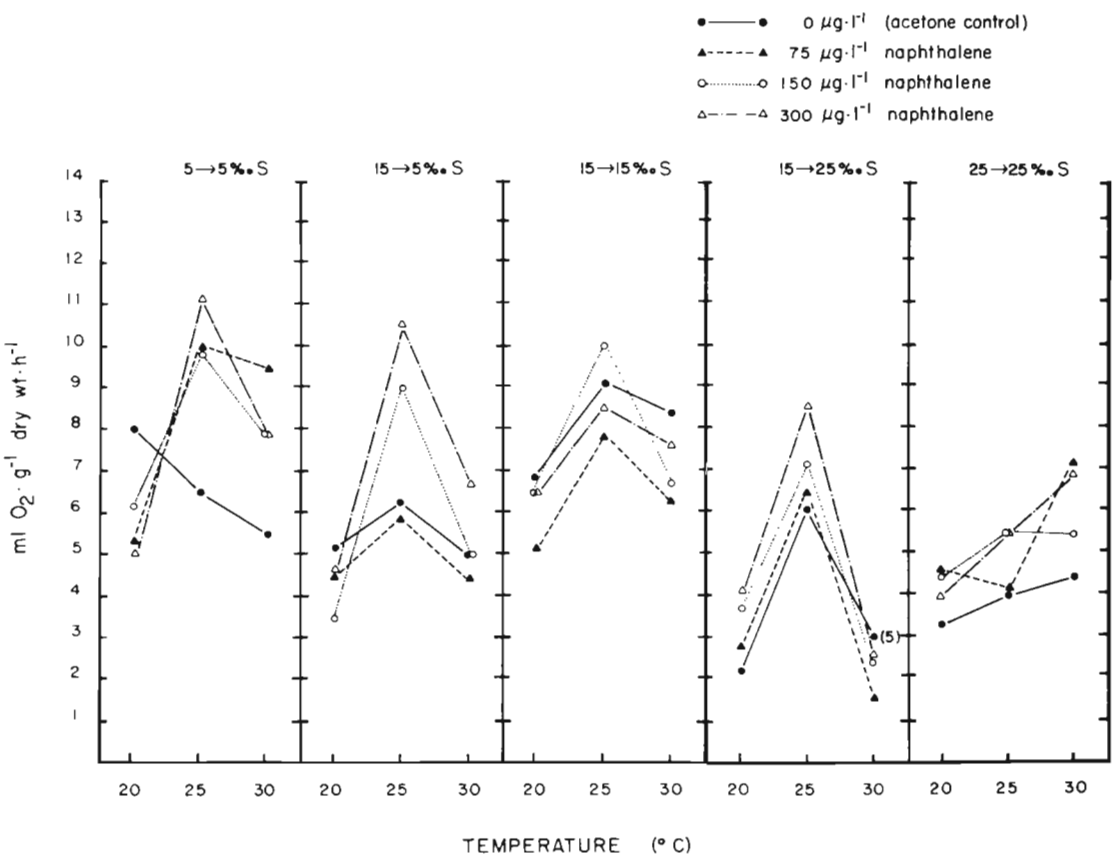

Fig. 1. *Rhithropanopeus harrisii*. Respiration rates of second zoeae under various factor conditions and exposure to naphthalene. Each point represents the mean of N = 6 determinations unless otherwise indicated by numbers next to the point

of 30 °C appeared to be particularly stressful since the lowest naphthalene concentration produced the largest increase over control respiration rates (two tailed t test: $T = -3.137$; $df = 10$; $0.02 > p > 0.01$). The two highest naphthalene concentrations caused larvae to have respiration rates intermediate in value between the control and low hydrocarbon-exposure rates.

A salinity of 15 ‰ seemed to bestow the greatest accommodation to hydrocarbon exposure in the early zoeae. While the respiration rates of exposed larvae remained depressed below the control value at 20 °C, the differences were small, particularly at high hydrocarbon levels. At 25 °C, the respiratory response was not dose-dependent. The intermediate hydrocarbon concentration (150 µg l^{-1}) produced a rate above the control while at the high and low levels (75 and 300 µg l^{-1}) mean respiration rates fell below the control measurement. The response at 30 °C for this salinity was similar to that at 20 °C with naphthalene exposure causing a decrease in mean respiration rates compared to that of the controls.

At 25 ‰ S, the mean respiratory response of naphthalene-exposed second zoeae was consistently above control values at all temperatures. At 25 °C, the response was dose-dependent. At 20° and 30 °C, the response was more variable in relation to hydrocarbon concentration. At 30 °C, the effects of hydrocarbon exposure were most marked.

The effects of hyperosmotic shock (transfer from 15 to 25 ‰ S) were the most extreme at high temperature, where there was a marked decrease in the mean respiration rates compared to the steady-state response of naphthalene-exposed animals at either 15 or 25 ‰ S. Control values at 30 °C decreased slightly from the steady-state response at 25 ‰ S. The respiratory rates for salinity-shocked second zoeae at 25 °C were intermediate in value between those observed for steady states at either 15 or 25 ‰ S. Their pattern, though, resembled that of the 25 ‰ S steady-state group. At 20 °C, the range of values and relationship was similar between the hyperosmotic shock groups and the 25 ‰ S steady-state group. At 20 °C, the range of values and relationship were similar between the hyperosmotic shock groups and the 25 ‰ S steady-state groups.

Hypoosmotic shock (transfer from 15 to 5 ‰ S) also produced the greatest effect at 30 °C. Here, most of the naphthalene-exposed groups had mean respiration rates greater than the mean rate for controls. The steady-state response at 5 ‰ S for these groups also was above the control rate. At 25 °C, 5 ‰ S steady-state and hypoosmotic-shock rates were similar except at 75 µg l^{-1} naphthalene, where the rate was lower for the osmotic shock group. Increase in respiration rate was dose-dependent at the higher concentrations. At 20 °C, hypoosmotic shock values for naphthalene-exposed animals were slightly lower than those recorded for the steady state, at 5 ‰ S, 20 °C. They were below control rates, as observed for the steady-state conditions.

Table 1 is a summary of the multiple regression analysis for the respiration rates of the second zoeae. Temperature and naphthalene exposure exerted significant main effects, and naphthalene exposure produced significant two-way interactions with both temperature and salinity. Other significant two-way interactions occurred between naphthalene exposure x osmotic shock, and salinity x osmotic shock. Both of the three-way interactions tested accounted for a significant portion of the observed variance. The relation of the data to the regression model used was fairly good ($r = 0.78$).

Fourth Zoeae

The fourth zoeal stage of this species is the last one before metamorphosis. Due to growth, the size of these larvae was much greater than that of second zoeae. Consequently, overall weight-specific oxygen consumption decreased owing to size effects. Additionally, continued naphthalene exposure produced differences in the respiratory response of this stage compared to the earlier one. For instance, at 15 ‰ S and 20 °C, the late zoeae exhibited a dose-dependent increase in the respiration rate (Fig. 2). The early zoeae showed a decrease relative to control rates. At 25 °C and 30 °C (15 ‰ S), the fourth zoeae had elevated respiration rates at high naphthalene levels. The opposite effect was evident at least at 30 °C for second zoeae. There was still an obvious salinity effect for fourth zoeae, with overall mean respiration rates tending to decrease with increasing salinity.

Crab larvae reared and exposed to naphthalene at 5 ‰ S had depressed respiration rates relative to control values at 20° and 30 °C with one exception at 30 °C, 300 µg l^{-1} naphthalene. There, the respiration rate was slightly elevated over that of controls. The effects of naphthalene exposure at 25 °C were variable; in general, respiration rate increased relative to that of controls, the highest naphthalene level producing the greatest increase.

The effects of higher-salinity (25 ‰) acclimation and naphthalene exposure produced a decrease in respiration rates at 20 °C, in a fashion similar to that observed at 5 ‰ S, 20 °C. At 25 °C, the low naphthalene levels caused an increase in respiration rates, but at 300 µg l^{-1} naphthalene, the mean rate fell slightly below the control value. Response at 30 °C, 25 ‰ S was similar to that at 30 °C, 15 ‰ S in that respiratory rates of

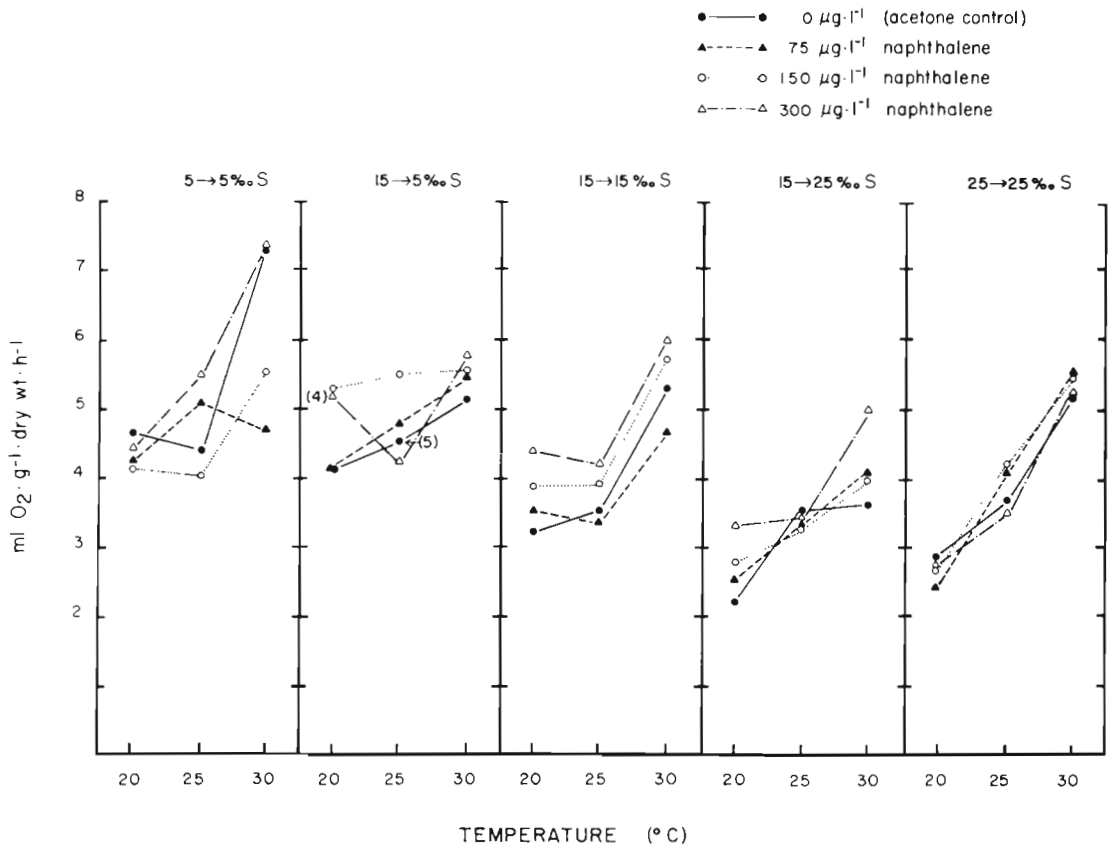

Fig. 2. *Rhithropanopeus harrisii*. Respiration rates of fourth zoeae under various factor conditions and exposed to naphthalene (N = 6 determinations unless otherwise indicated)

exposed larvae were elevated slightly over control values. The opposite trend occurred at 20 °C. However, the differences among the means at each temperature at 25 ‰ S were small compared to those for the other salinities tested.

Although the actual mean respiratory rates were similar in value, hypoosmotic shock caused by a move from 15 to 5 ‰ S reversed the trend of the respiratory pattern compared to the steady-state response at 5 ‰ S. Except for one instance (25 °C, 300 μg l^{-1} naphthalene), the rates for exposed animals increased over control values, particularly at high naphthalene concentrations.

The salinity effect was obvious when the animals were transferred from 15 to 25 ‰ S immediately prior to respirometry. At both 20° and 30 °C there was a dose-dependent increase in respiratory rates, but at 25 °C the mean rates were below the mean for controls in a dose-dependent pattern. Differences were not large.

The multiple regression analysis given in Table 1 shows that compared to the second zoeae, the fourth zoeae displayed fewer significant interactions among variables affecting the respiration rates. Of the two-way interactions tested, only temperature x salinity was significant. Notable for this stage, neither the linear or quadratic effects of naphthalene exposure accounted for significant portions of the variance associated with respiration rates, as assessed by the statistical model.

Megalops

There were few differences between the general pattern of megalopal respiration rates and those of zoeal stages. However, the effect of salinity was much smaller than in earlier stages. There appeared to be an increasing tolerance to naphthalene exposure, only the highest naphthalene levels producing a marked change in respiratory rates compared to those of controls (Fig. 3).

The effect of naphthalene exposure was most pronounced in the steady-state response at 5 ‰ S. Under both 20° and 30 °C temperature regimes, the highest naphthalene level, 300 μg l^{-1}, produced marked increases in respiratory rates. At 25 °C, exposure to 300 μg l^{-1} naphthalene caused a decrease in the respiration rate. The effect of intermediate naphthalene concentrations was minimal.

Mean respiratory rates of megalopae at 15 ‰ S

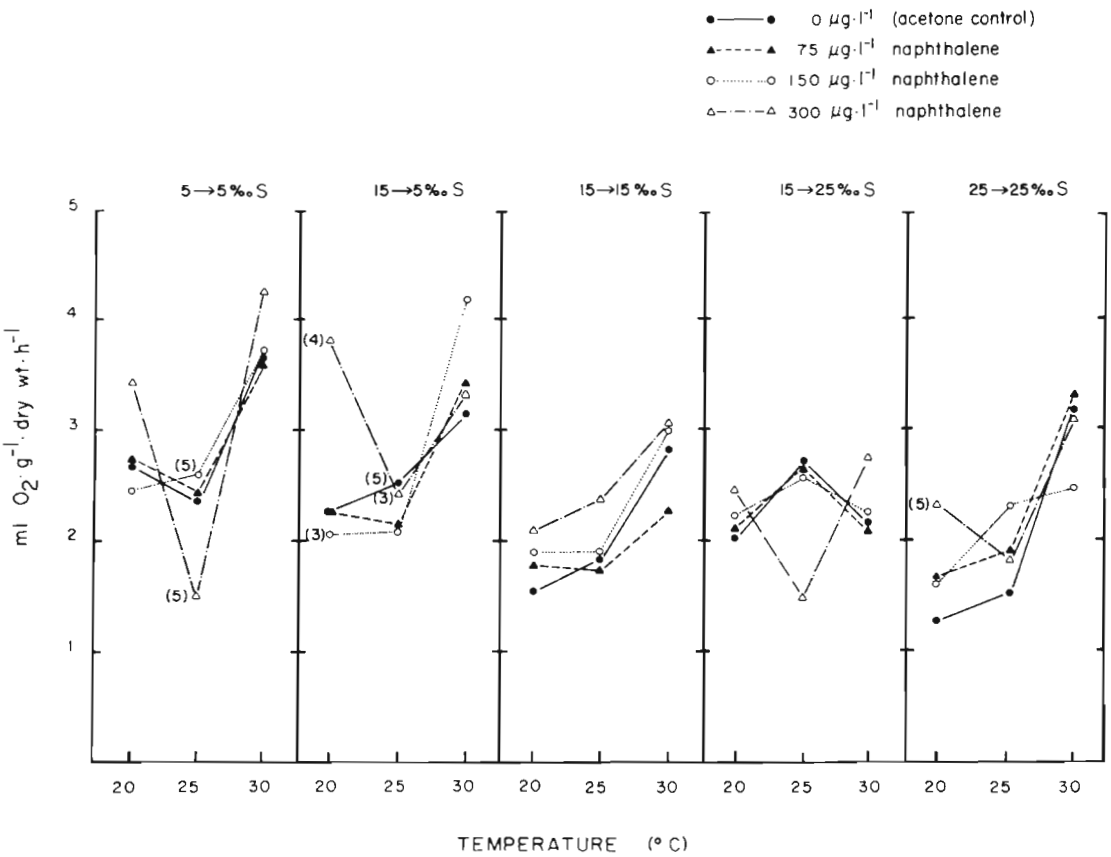

Fig. 3. *Rhithropanopeus harrisii*. Respiration rates of megalops under various factor combinations and exposed to naphthalene (N = 6 determinations unless otherwise indicated)

increased with temperature. At 20 °C, there was a dose-dependent increase in the rates over the control. At the two higher temperatures, the two highest naphthalene concentrations caused dose-dependent increases over control rates, but at 75 µg l^{-1} naphthalene the mean rates were below those of controls.

The steady-state respiratory response at 25 ‰ S was not affected consistently by naphthalene exposure at any temperature. At 20° and 25 °C, exposed animals exhibited mean respiratory rates above that of the control, but the response was not dose-dependent. At 30 °C, the two highest naphthalene levels caused mean respiratory rates to decrease below control rates, and at 75 µg l^{-1}, the mean rate was elevated slightly over that of controls.

Compared to the steady-state response at 5 ‰ S, hypoosmotic shock had the effect of lowering the overall respiratory rates at 20° and 25 °C, causing them to decrease at low and increase with intermediate naphthalene levels at 30 °C. The effects were not dose-dependent at any temperature.

The effect of hyperosmotic shock, compared to the steady-state response at 25 ‰ S, was greatest at 25 °C where there was a dose-dependent decrease in mean rates with increasing naphthalene concentration. At both 20° and 30 °C, the differences between the exposed and control rates were greatly diminished compared to the 25 °C steady-state values. There was a dose-dependent increase over control rates at 20 °C for all naphthalene concentrations, and for the two highest ones at 30 °C.

The results of the multiple regression analysis would suggest that the megalops is a fairly sensitive stage, at least with respect to the effect of the physical factors tested (Table 1). There was a significant three-way interaction between temperature, salinity and osmotic shock. However, once again there was no significant overall effect of naphthalene on the respiratory rates, nor were interactions between naphthalene exposure and physical factors significant.

The respirometry data for megalops were suitable for response surface analysis (Fig. 4). Under steady-state salinity conditions, the center of the response surface, a minimum, occurs within the environmental ranges of salinity and temperature used. The predicted minimum was centered at about 27 °C and 22 ‰ S. Naphthalene exposure did not change the ranges of temperature and salinity where respiration rates were

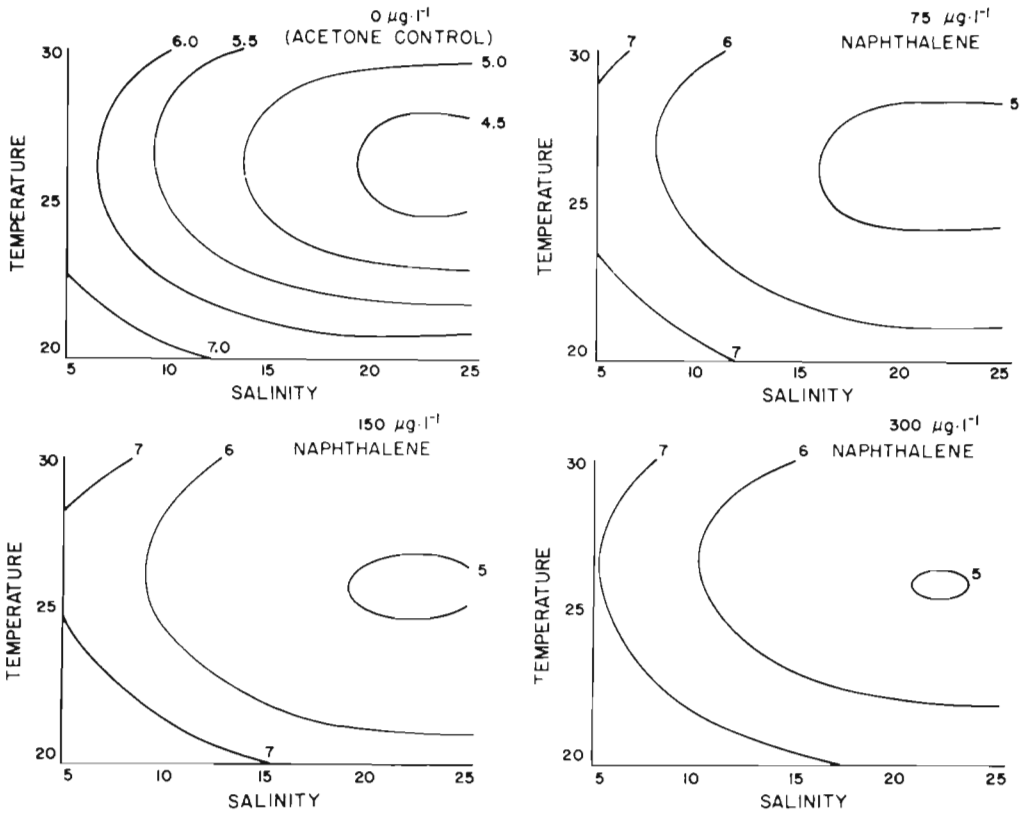

Fig. 4. *Rhithropanopeus harrisii*. Predicted megalopal respiration rates at steady-state salinities and different temperatures and naphthalene-exposure concentrations (N = 6 determinations unless otherwise indicated)

at a minimum, but overall, it did cause increased respiration rates. This naphthalene-mediated increase was greatest at low salinities. Response surfaces of the predicted respiration rates of megalops during osmotic shock (not shown) were similar in shape to those for the steady state.

First Crab Stage

On reaching the first crab stage, the proportional changes in weight with stage had begun to diminish. Consequently, differences due to weight in weight-specific oxygen consumption became less obvious when compared to the data for the megalops. Furthermore, the effects of salinity on the overall respiration rates were much less. Only the lowest salinity, 5 ‰ S, appeared to produce a continued, marked salinity effect (Fig. 5). This pattern persisted through at least the juvenile stages tested elsewhere with phenanthrene (Laughlin and Neff, 1980).

At the low salinity, the highest naphthalene exposure concentration always caused mean respiratory rates to be above control levels for that temperature. However, there was a strong temperature effect at low and intermediate naphthalene levels. At both 20° and 30 °C, these mean rates were above control levels; at 25 °C, they were below those of the control.

Animals acclimated to 15 ‰ S and tested at that salinity exhibited reduced respiration rates, relative to control values, only at 20 °C. At both 25° and 30 °C, there was an increase in respiration rates due to naphthalene exposure, with a prominent increase at 30 °C, 300 µg e^{-1} naphthalene. At 25° and 30 °C, increases were not consistently dose-dependent.

At the highest acclimation salinity (25 ‰ S) and a temperature of 30 °C, naphthalene exposure resulted in a decrease in respiration rates relative to those of the control. The opposite was observed at 25 °C. At 20 °C, low concentrations of naphthalene had no effect on mean respiration rates, but at 300 µg l^{-1} naphthalene the mean rate was elevated over the control rate.

Hypoosmotic shock caused by a move from 15 to 5 ‰ S appeared to have its greatest effect at the highest naphthalene concentration and at the high and intermediate temperatures, 25° and 30 °C. There was a marked increase in respiration rate over the control rates under these conditions, the greatest differences occurring at 30 °C. Only at 25 °C did the increase over control rates occur for all naphthalene concentrations.

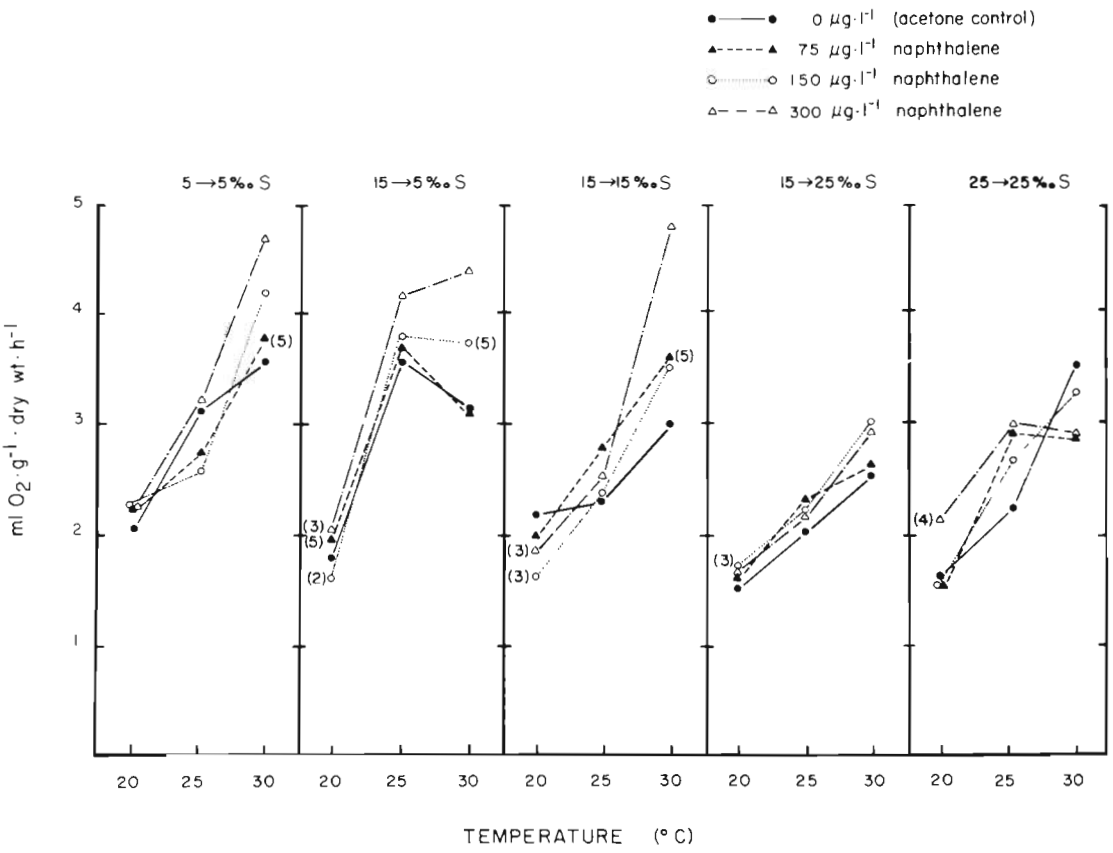

Fig. 5. *Rhithropanopeus harrisii*. Respiration rates (ml $O_2 \cdot g$ dry wt^{-1} h^{-1}) of the first crab stage under various temperature-salinity conditions when exposed to naphthalene

At 20 °C, the effects of naphthalene exposure on the mean rates were small.

When the first crabs were given hyperosmotic shock, the naphthalene-exposed groups always had mean respiration rates above control rates. However, the differences among the means were not large nor were they consistently dose-dependent at any temperature. In general, the values observed were similar to those determined for first crabs at steady state in 25 ‰ S.

Respiration rates of the first crab stage (Table 1) suggest this stage is more tolerant to salinity and osmotic shock. Notably, there was a marginally significant effect of naphthalene exposure on respiratory rate. Particularly important in this regard was the three-way interaction between temperature, salinity and naphthalene exposure. Also, there was a significant two-way interaction between temperature and naphthalene exposure.

The response surfaces for the acclimated respiratory rates of the first crab stage exposed to naphthalene (Fig. 6) are similar to those observed for the megalops, although there are two obvious differences. First, the crabs exhibited a very broad range of tolerance with respect to both physical factors tested. Second, the centers are shifted somewhat from those observed for the megalops. Optimal salinities occur near 18–20 ‰ S. The temperature optimum is shifted to about 27 °C, a slight increase over that observed for megalops. The general result of exposure to naphthalene was an increase in predicted respiration rates. The increase was particularly prominent at low salinities.

Megalopal Weights

Mean weights of megalops reared under the different treatment combinations (Fig. 7) ranged from 139.7 to 235.3 µg megalops^{-1}. There was a great deal of variability within and among the different groups. Reduced weights were associated with both low salinities and high temperatures. The effects of naphthalene exposure were not as obvious, except where the physical conditions were suboptimal, particularly at low salinities and high temperatures. For instance, the two groups with the lowest mean dry weights occurred at the intermediate and highest naphthalene concentrations at 5 ‰ S, 30 °C. The analysis (Table 2)

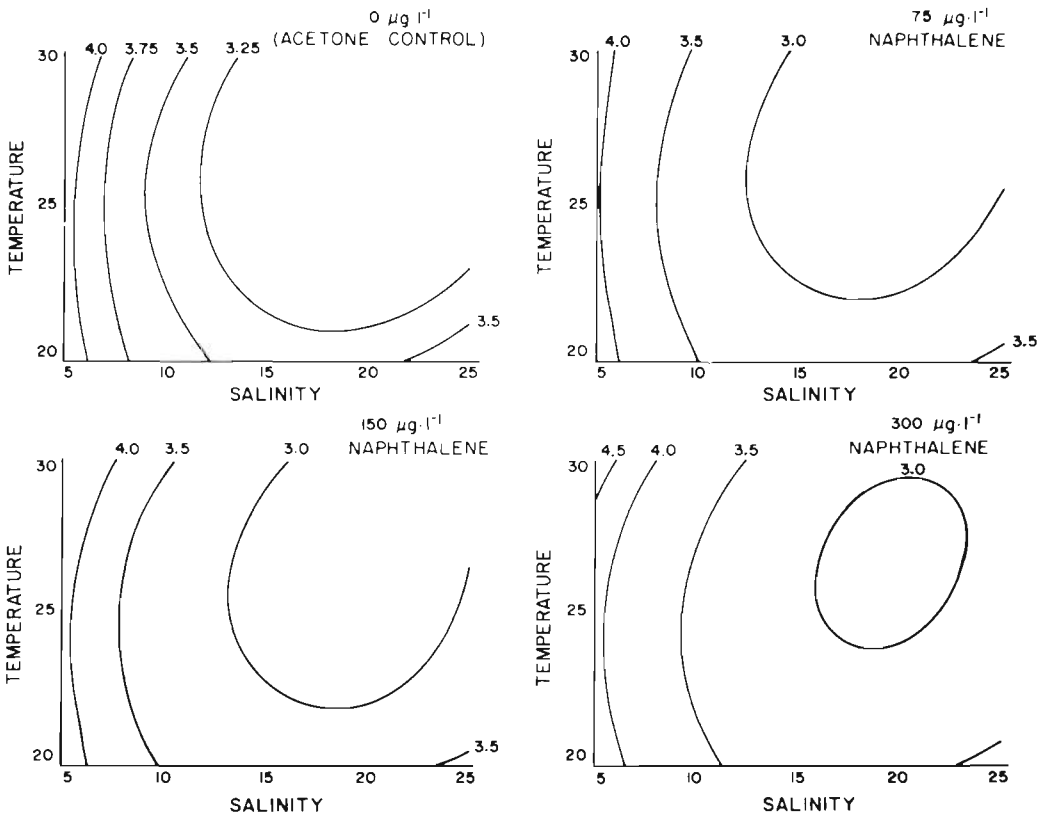

Fig. 6. *Rhithropanopeus harrisii*. Predicted respiration rates (ml 0_2 g dry wt^{-1} h^{-1}) of the first crab stage at steady-state salinities and different temperature and naphthalene-exposure concentrations

Table 2. *Rhithropanopeus harrisii*. Synopsis of the multiple regression analysis of the effects of temperature (T), Salinity (S), and naphthalene exposure (N) on megalopal weights

Correlation coefficient	Significance of F statistic		
	≥.10	.05 > F > .01	≤.01
0.76	N^2	N, T × S	T, S, T^2, S^2, T × N, S × N, T × S × N

indicates a significant three-way interaction among the three factors tested. All two-way interactions were significant.

Response surfaces predicting the dry weights (Fig. 8) clearly indicate the effects of both low salinity and high temperature. Although the main effect of naphthalene exposure was statistically insignificant, the response surfaces indicate a trend toward increases in megalopal size with increasing dose. The differences in the predicted sizes in this case are small, i.e., 225 µg megalops^{-1} at 0 ppb naphthalene and 242 µg megalops^{-1} in 300 µg l^{-1} naphthalene. In contrast, the contours predict a much greater influence of temperature and salinity on the weight of the megalops.

DISCUSSION

The data show that increases in temperature and decreases in salinity are accompanied by relative increases in respiration rates throughout the larval development of the mud crab *Rhithropanopeus harrisii*. Unlike phenanthrene exposure (Laughlin and Neff, 1980), naphthalene exposure was not as great an effector of increased respiration rates as were temperature and salinity, and its effect declined with age suggesting metabolic compensation, perhaps related to detoxification (Lee, 1975). Concomitant with increases in respiration rate, the weights of megalops reared at high temperatures and low salinities were lower than those of crabs reared under optimal conditions. A comparison of the response surfaces for the megalops shows that in both cases the lowest respiration rates and highest growth rates were centered near 22 °C and 20 ‰ S, indicating that the larvae are well-adapted to life in the mesohaline estuaries where they occur naturally.

Respiration rates were measured under osmotic shock conditions to determine qualitative and quantitative effects of salinity changes that might occur under natural conditions. While metabolic tempera-

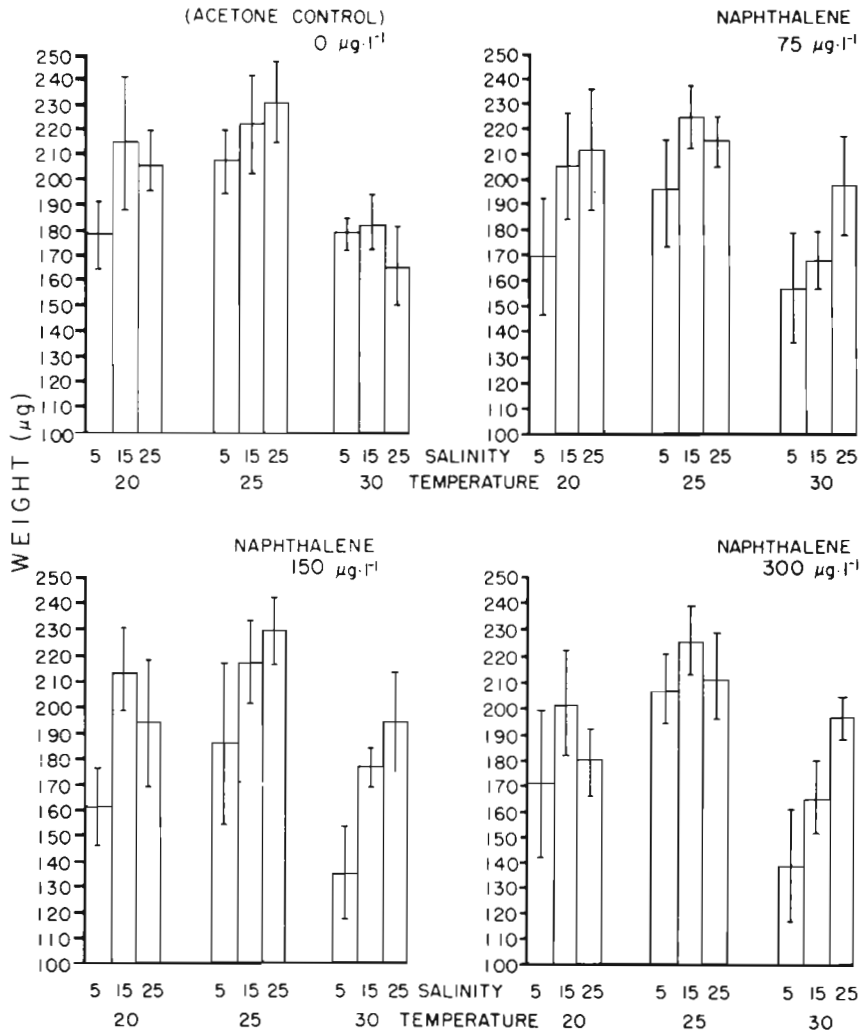

Fig. 7. *Rhithropanopeus harrisii*. Weights of megalops from zoeae reared under various combinations of temperature, salinity and naphthalene exposure concentrations

ture compensation has been studied extensively, salinity acclimation is only now receiving increased attention. Our data for all stages indicate that acclimation to decreasing salinity produces greater changes in respiration rates than a comparable change to higher salinities. The process of larval development leading to metamorphosis in crustaceans imposes some constraints on the strategies of osmotic regulation compared to the adults.

Although most stages demonstrated increased respiration rates with increasing temperature, the Q_{10}'s for the interval from 20° to 30 °C were usually between 1 and 2, indicating a relative degree of metabolic temperature independence. Somero and Hochachka (1976) and Somero (1978) have suggested that changes may occur in the tertiary structure of enzymes with temperature. Such changes may involve the strength of weak bonds, such as hydrogen bonding, between the protein and the solvent (water), and modify the affinity of the enzyme for its substrate. Consequently, changes in catalytic activity with temperature tend to yield fairly constant reaction rates in spite of temperature change. This hypothesis makes no distinction as to the degree of ontogenetic development, and none was observed in *Rhithropanopeus harrisii*. Because of the antagonistic relationship between carbon respired and assimilated for the rapid growth characteristic of larval development, temperature compensation should be very important. Thus, it is not surprising that some degree of temperature compensation occurs in all life stages.

The relationship between increased respiratory loss of carbon and decreased growth is intuitively obvious. However, the role of salinity as a metabolic mediator is less clear. Adaptation to salinity change is an active process sensitive to both levels and gradients of change (Kinne, 1964). It has been shown that both

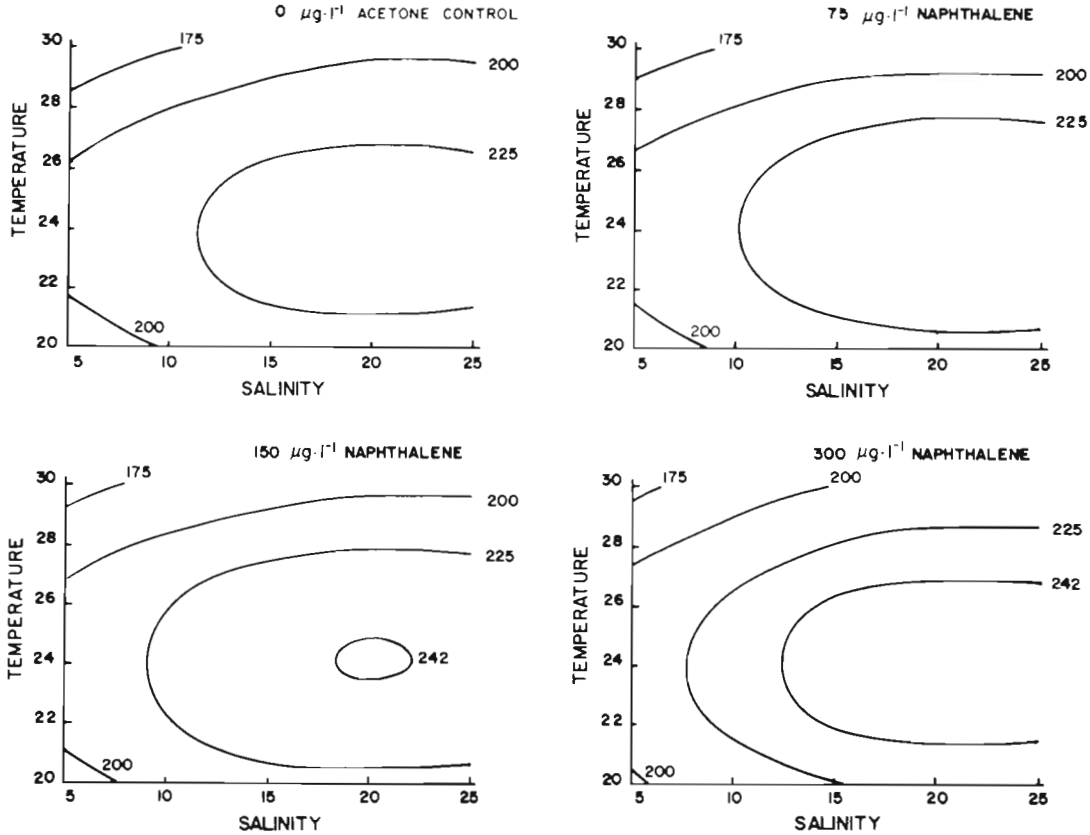

Fig. 8 *Rhithropanopeus harrisii*. Predicted dry weights (µg) of megalops from zoeae reared under various combinations of temperature, salinity and naphthalene exposure concentrations

zoeal stages (Kalber and Costlow, 1966) and the crab stages exhibit increasingly strong hyperosmotic regulation as salinity decreases, and the crab stages regulate chloride ion concentrations between 275 and 375 meq l^{-1} in external salinities between 5 and 30 ‰ S (Laughlin and Neff, 1979b). Thus at least a part of the increased respiration rates at low salinities may reflect active ionic and osmotic extra-cellular regulation.

There is abundant evidence showing correlations between changes in respiration rate of whole animals or tissues with changes in salinity (Dehnel, 1960; Dehnel and McCaughran, 1964; King, 1965, 1966; Engel and Eggert, 1974; Engel et al., 1975; Dimock and Groves, 1975). In most of the studies quoted it was assumed that changes in respiration rates with salinity should be reflecting energetic costs of homeostasis, yet no consistent estimate of osmoregulatory cost can be made from the data. There have been few attempts to determine the cost of ionic regulation *per se;* the few available yield low values. For instance, Bielawski (1971) estimated that only about 3 % of the energy budget of the freshwater crayfish *Astacus* is committed to ion regulation. This is not enough to account for megalopal weight differences of 10 % or greater due to salinity observed in this study.

Because the amount of energy required for ion translocation is so small, as a proportion of total metabolism, it is likely that salinity effects on metabolism and growth would be exerted through salinity-dependent changes in intracellular amino acid levels (Florkin and Schoffeniels, 1965, 1969; Costlow and Sastry, 1966; Schoffeniels and Gilles, 1970; Gerard and Gilles, 1972). These are known to affect the respiration rates of whole animals or specific tissues (Hulbert et al., 1979a, b). Quantitative estimates of the metabolic cost of intracellular isosmotic regulation based on our data would be speculative, but qualitative inferences suggest that it may be an important energetic component of homeostasis.

The respiratory response to osmotic shock also would be mediated to a large extent by the specific mechanisms of adaptation. Based on asymmetric time-dependent osmotic regulation characteristics, it is probable that different strategies are employed for hyperosmotic and hypoosmotic stress (Findley et al., 1978). One indicator is evident by comparing the steady-state and osmotic-shock responses. Generally, rates determined during hypoosmotic shock resembled the steady-state response at 5 ‰ S, but those caused by hyperosmotic shock were intermediate between the

ones measured at 15 and 25 ‰ S. This may be an artifact of experimental design if only the adaptation rate was different for the two stresses. However, Dimock and Groves (1975) have reported similar interactions between changing temperatures and salinities on the respiration rates of adults of the mud crab species *Panopeus herbstii*.

Effects of Naphthalene

Naphthalene was chosen as a model toxicant for these experiments because it is structurally the simplest in a family of chemicals known to be largely responsible for the toxicity of crude and refined oils (Anderson et al., 1974). Naphthalene is characterized by relatively rapid uptake-depuration kinetics (Neff et al., 1976). Detoxification systems, present in many invertebrate phyla, metabolize it (Lee et al., 1977; Neff, 1979), although they may not be as efficient in larval forms as they are in adults (Sandborn and Malins, 1977). Finally, the effects of naphthalene on physiological functions, e.g. crustacean respiration rates (Tatem, 1977) have been shown to be reversible following depuration of tissue burdens of naphthalene isomers accumulated during exposure to No 2 fuel oil water-soluble fractions.

Because naphthalene concentrations used in this experiment were not acutely toxic, changes in respiration rate caused by aromatic hydrocarbon exposure can be attributed to two different modes of action. The first effect of naphthalene would be narcosis (Crisp et al., 1967), which would affect oxygen consumption indirectly by decreasing activity. The second would occur at the subcellular level, and has been most clearly demonstrated by Percy (1977). He found that respiration rates of mitochondria isolated from oil-exposed amphipods increased significantly over controls. The apparent lack of naphthalene's dose-dependent effect can be attributed to these antagonistic modes of action. Narcosis would be the predicted major effect either in low exposures, or early in the course of exposure when tissue burdens were relatively low. When exposure levels and tissue burdens were high, or before the induction of effective metabolic degradation/depuration pathways were effective, accelerating effects on oxygen consumption would occur. The ontogenetic effect appears to be a decrease in the narcotic effect and an increase in metabolic rates.

Recently, Johns and Pechenik (1980) examined all components of the energy budget of *Cancer irroratus* larvae exposed to 'water-accommodated fractions' of No. 2 fuel oil. They found effects on respiration and growth similar to ours. They also reported lower feeding rates in oil-exposed larvae, perhaps a narcosis effect. To our knowledge, however, there are few reports of the effect of environmental variables on components of the energy budget altered by petroleum hydrocarbon exposure. The data presented here clearly indicate that these are important factors influencing the relative contribution of narcosis versus metabolic acceleration as a response to low environmental petroleum hydrocarbon concentrations.

The growth response to naphthalene also exhibited a somewhat contradictory pattern. Although low salinity-high naphthalene combinations reduced growth rates, exposure in optimal conditions appeared to have little retarding effect, and the statistical model even predicts a slight enhancement of growth rates. This enhancing effect has been observed before in various 'stress' situations and has been attributed to a phenomenon known as 'sufficient challenge' (Smyth, 1967) or 'hormesis' (Stebbing, 1979) and has been attributed to over-corrections in homeostatic mechanisms caused by stress well within a range that can be counteracted.

The partial energy budget approach used to assess sublethal stress in developing crab larvae has proven to be a sensitive method. The interaction of pollutional, physical and ontogenetic factors producing sublethal stress are causally related to the extent that a response at one level of a factor is mediated by the levels of other factors involved. Alderdice (1972) has pointed out though that these relationships cannot be inferred through univariate approaches; they must be measured experimentally. In laboratory studies, it is essential that these factors be considered so that realistic judgements of the biological effects of pollutants can be estimated.

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