

IONIC AND OSMOTIC REGULATION, METABOLIC RESPONSE TO
SALINITY, AND PHYSIOLOGICAL RESPONSE TO PESTICIDES OF JUVENILE
CALLINECTES SAPIDUS RATHBUN

By

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Abstract of Dissertation Presented to the Graduate Council
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The osmotic and ionic regulation and the metabolic response to salinity of juvenile *Callinectes sapidus* Rathbun were investigated. Juvenile blue crabs at 25°C hyper-osmoregulate in medium of less than 700 mOs/l and osmoconform at higher salinities. Hemolymph Na⁺ and Cl⁻ concentrations increase gradually with increasing external concentrations below 700 mOs/l and increase more rapidly at higher concentrations: only Cl⁻ is hypo-regulated. The internal K⁺ concentration is higher than the external concentration at all external concentrations tested (as high as 15 meq/l). At 16°C and concentrations less than 700 mOs/l total hemolymph concentrations are higher than at 25°C. The metabolic rates of juvenile blue crabs acclimated to test concentrations between 50 and 1410 mOs/l are not significantly different. Abrupt transfer from a higher acclimation concentration to a lower concentration results in a large increase in the metabolic rate. At O₂ concentrations greater than 2.0 ml O₂/l juvenile blue crabs are metabolic regulators.

DDT and Mirex concentrations in juvenile blue crabs from the Cedar Key, Florida, U.S.A. estuarine zone were low. The effects of ingested DDT and Mirex on the physiology of juvenile blue crabs were examined. The crabs are sensitive to these pesticides when the compounds are ingested--to Mirex more than to DDT. High, subacute internal levels of DDT and Mirex result in pronounced metabolic rate elevations, reduction in critical oxygen concentration, inhibition of the autotomy reflex (Mirex), and reduced carapace thicknesses. DDT and Mirex below acute levels do not affect patterns of osmotic and ionic regulation. I conclude that DDT and, to a far greater extent, Mirex are potentially disastrous agents with respect to blue crab populations.

INTRODUCTION

The blue crab, *Callinectes sapidus* Rathbun, is an economically significant resident of Atlantic estuarine zones. In recent years the numbers of these animals along the southeastern U.S.A. coasts have declined (Mahood *et al.*, 1970). The success of populations will depend upon juvenile success which in turn depends upon their physiological responses to different environmental conditions.

Temperature and salinity are master environmental factors that are highly variable in estuarine zones. Some temperature effects on juvenile blue crabs have previously been investigated (Leffler, 1972) but physiological responses to different salinities had not been studied. Recently, pesticides have also become notable environmental components of many estuarine zones. Numerous investigations into the acute toxicity of pesticides to estuarine animals have been conducted (*e.g.* Muncy and Oliver, 1963; Naqvi and Ferguson, 1968; Eisler, 1969; Macek and Sanders, 1970). The effects of subacute levels of pesticides on organisms as well as their acute toxicity are important. Subacute concentrations could disrupt physiological processes of individuals to the extent that continued prosperity of the population is impossible. Therefore, it is imperative to examine the consequences of chronic, subacute pesticide exposure on estuarine inhabitants.

This study on juvenile blue crabs was undertaken to determine:

1. patterns of hemolymph Na^+ , K^+ , Cl^- , and total osmotic particle regulation,

2. metabolic costs of osmotic regulation at different salinities,
and

3. effects of DDT and Mirex on the metabolic rate, ionic and osmotic regulation, ability to autotomize limbs, and carapace thicknesses.

MATERIALS AND METHODS

The experimental animals were juvenile blue crabs, *Callinectes sapidus* Rathbun, (0.75-2.6 g [dry], 35-50 mm [width]) of both sexes from the estuarine zone near Cedar Key, Florida, U.S.A. A commercial shrimp fisherman captured the crabs at night on sea grass flats during all seasons (1971-1974).

The crabs were maintained in the laboratory for 35 days before physiological measurements were made. The tanks were 103 liter aquaria divided into 6 chambers. Water was circulated and filtered through activated charcoal by a power filter maintaining an O_2 concentration above 4.0 ml/l in the acclimation water. The full sea water (approximately 1000 mOs/l [34 ‰]) was filtered ocean water from Marineland (Marineland, Florida, U.S.A.). It was made more saline by the addition of synthetic marine salt mix (Instant Ocean) or diluted to the desired concentrations. The concentrations were measured with an Osmette precision osmometer. Coquina (*Donax variabilis*) shell fragments were placed on the floors of the tanks. The water was replaced and the substrate rinsed after the experiments on each group of crabs were completed.

Two acclimation temperatures $16^\circ C (\pm 1^\circ)$ and $25^\circ C (\pm 1^\circ)$ were maintained. The photoperiod was 14h light and 10h dark.

The diet consisted of three feedings weekly (on alternate days) of one 3 mm^3 piece of beef liver or shrimp per crab. Feeding was discontinued 5 days prior to the beginning of the actual experimentation.

Mirex (dodecachlorooctahydro-1, 3, 4-metheno-2H-cyclobuta [cd] pentalene) and p,p'-DDT (1, 1, 1-trichloro-2, 2-bis [p-chlorophenyl] ethane) (chromatographically pure) were administered orally once per week for a total of 2, 3, or 4 feedings. Food items were soaked in acetone solutions of pesticides for measured lengths of time at 2°C. The treated food was frozen until used. Food type, particle sizes, solution concentrations, soaking times, and the resultant pesticide concentration of the food are given in Table 1. The treated food particles were placed directly against the crab's mouth parts and were devoured within a couple of minutes. Control groups were run simultaneously with experimentals. One control group received food soaked in acetone at one feeding each week while a second received only untreated food. As no physiological differences were observed between these two groups, they are combined and are considered untreated crabs in all further discussion. Metabolic rate determinations were made 7 days after the last treatment--one untreated feeding being given after the final treatment.

Metabolic rates were measured by determining O₂ uptake in closed chambers. The chambers were opaque Erlenmeyer flasks fitted with rubber stoppers pierced by three hypodermic needles. One needle was fitted with catheter tubing that reached to the bottom. When the flask was filled with water this arrangement allowed water samples to be taken from the top and the bottom. A single, post-absorptive, intermolt (stage C) crab was placed in each aerated chamber that was, in turn, placed in a water bath maintained at the desired temperature. After the crab became quiet, the aeration was terminated, the chamber sealed, and water

Table 1. The preparation and composition of food items utilized for pesticide treatment.

Group	Food material	Approximate particle wt. (g)	Pesticide concentration in soaking solution ($\mu\text{g/ml}$)	Hours soaked	Mean wt. of pesticide per piece of food (μg)
DDT ₁ *	liver	0.08	10	33	0.53**
DDT ₂	shrimp	0.10	30	40	3.2
Mirex ₁	shrimp	0.02	5	24	0.03
Mirex ₂	shrimp	0.02	5	33	0.06
Mirex ₃	liver	0.04	5	33	0.14
untreated	shrimp	0.02	0	33 or 0	DDT family--0.018 Mirex --0
untreated	liver	0.04	0	33 or 0	DDT family--0.041 Mirex --0

*The subscript is solely a means of identification of the quantity of pesticide in the treatment. This method of labeling will be used to identify the amount of pesticide given to the crabs throughout this report.

**DDT concentrations are given as total of DDT family (*i.e.* DDT, DDD, DDE).

samples were taken at hourly intervals for 3 h. Oxygen concentrations of the samples were determined using a Radiometer pO_2 electrode. At the conclusion of the test the crabs were removed from the chambers, weighed, and returned to their acclimation treatment tanks. All metabolic determinations were made between 10 A.M. and 4 P.M.

Two days after the metabolic rate measurements were made concentrations of Na^+ , K^+ , Cl^- , and total osmotic concentrations of the hemolymph of the same individuals used for metabolic experimentation were measured. Samples were obtained by piercing the arthrodistal membrane at the base of the fifth walking leg with a glass capillary pipette. Hemolymph concentrations were determined with a Clifton nanoliter osmometer or on pooled samples with an Osmette precision osmometer. The hemolymph ionic composition was analyzed using a Radiometer Model FLM 2 flame photometer (Na^+ , K^+) and a Radiometer Model CMT 10 chloride titrator.

The capacity of each crab to autotomize a damaged limb was determined by crushing the merus of the fourth walking leg. The crab was then placed in hot water ($60^\circ C$). The extent of autotomization resulting from an extreme generalized stimulus was recorded. All crabs died within seconds and were removed. Crabs were dried at $105^\circ C$ for 24 h. Twenty minutes after removal from the oven they were weighed. Carapace widths and thicknesses (mean of three mid-dorsal measurements) were measured with calipers.

Chlorinated hydrocarbon levels were determined using a Varian Aerograph, Model 600-D, gas chromatograph containing a 6' X 1/4" (OD) glass column of 1:1 6.4% OV-210:1.6% OV-17 on Chromosorb W and equipped with an H electron capture detector. The column and detector tempera-

tures were 215° and 218°C respectively. Carrier gas (N₂) flow rate was 45 ml/min. Crabs were analyzed in groups of four that had undergone similar treatment. Chlorinated hydrocarbons were removed from tissues by the following method: the crabs were ground along with anhydrous Na₂SO₄ in a Virtis "45" blender; the lipids and chlorinated hydrocarbons were extracted from the powder into 1 acetone: 2 hexane (Soxlet apparatus, 16 h); the sample was evaporated nearly to dryness; cleanup was made by placing the sample (dissolved in hexane) on a deactivated Florisil (10% water) column and removing the chlorinated hydrocarbons with a 3 hexane: 1 benzene emulsion. The sample was evaporated to about 5 ml and the volume increased, if necessary, with benzene for injection into the gas chromatograph. Samples of DDT, DDD, DDE, and Mirex of known concentrations were processed (Soxlet, Florisil column) with each three samples analyzed to ascertain the percentage recovery:

mean % recovery - DDT family (DDT, DDD, DDE) - 85.1 ± 9.6*

Mirex - 88.0 ± 5.5.

Pesticide concentrations reported have been corrected for the deviation from 100% recovery.

*Numerals following ± designate standard error of means.

RESULTS

Osmotic and Ionic Regulation

Juvenile blue crabs maintain hemolymph osmotic concentrations higher than all external concentrations less than 700 mOs/l (25°C) but crabs acclimated to lower salinities have lower hemolymph concentrations than those acclimated to higher salinities (Figure 1). For example, at an external concentration of 700 mOsm/l the hemolymph concentration is about 700 mOs/l but this drops to 575 mOs/l at 50 mOs/l.* Above 700 mOs/l, juvenile blue crabs at 25°C are approximately isosmotic with the environment (Figure 1).

Regulation of Na^+ and Cl^- follows similar patterns to that of total osmotic concentration (Figures 1, 2, and 3). Sodium ions are hyper-regulated up to an external concentration of about 325 meq/l (equivalent to a total osmotic concentration of 760 mOs/l). Within this hyper-regulatory range hemolymph Na^+ concentration increases in response to increases in external concentration. For example, at an external Na^+ concentration of 25 meq/l the hemolymph Na^+ concentration is approximately 280 meq/l but the hemolymph concentration increases to about 320 meq Na^+ /l at an external concentration of 300 meq Na^+ /l. Sodium ions are slightly hyper-regulated and conforming to external concentrations between 325 and 500 meq/l (about 1200 mOs/l). Hemolymph and external Na^+ concentrations are equal at 625 meq/l (1410 mOs/l). Hemolymph Cl^- con-

*All differences cited (hemolymph concentration, metabolic rate, etc.) are significant at 95% confidence (Student-t).

Figure 1. The effect of external osmotic concentration on the hemolymph osmotic concentration of juvenile blue crabs not treated with pesticides (o), DDT₁ treated (●), DDT₂ treated (*), and Mirex₃ treated (X) at 25°C and untreated juvenile blue crabs at 16°C (+). Lines are adult blue crabs from Gifford, 1962 (.....), Ballard and Abbott, 1969 (— — —), and Lynch *et al.*, 1973 (-----).

(Diagonal is equality.)

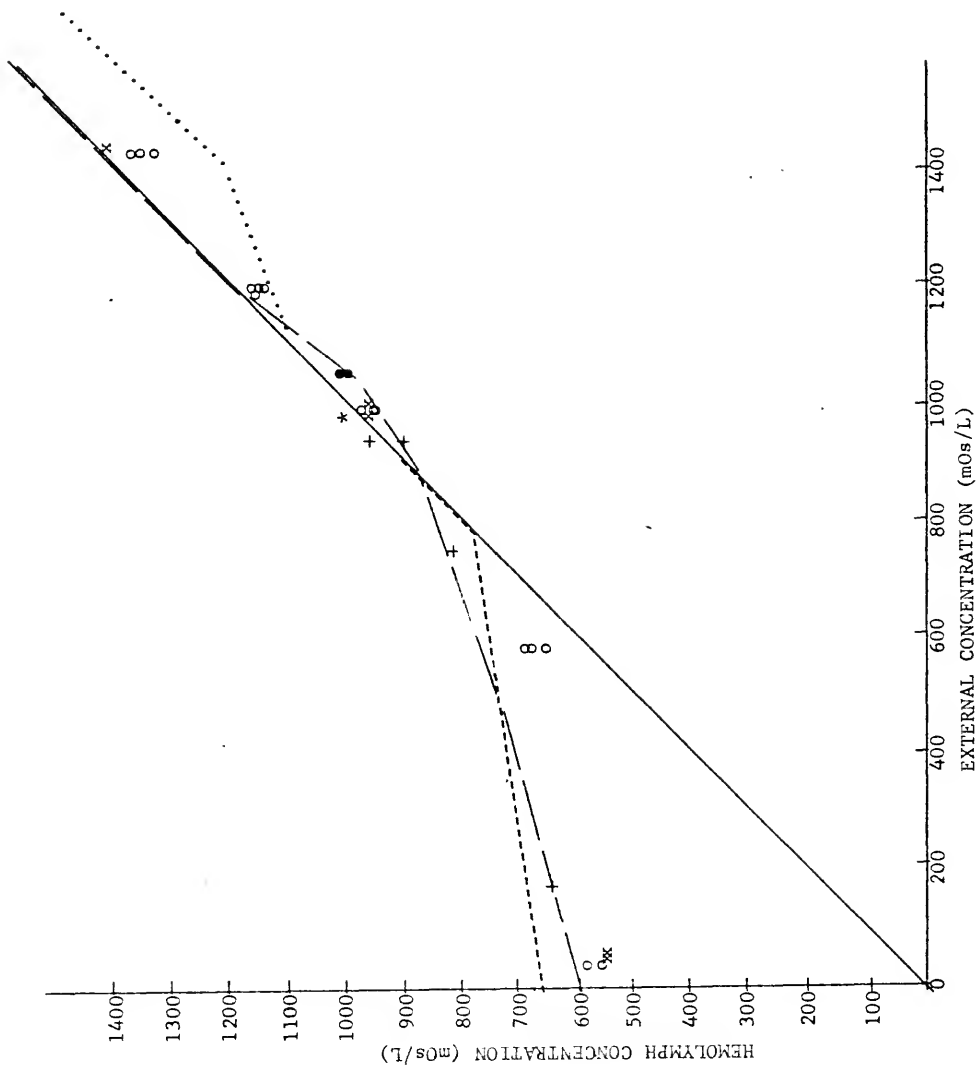


Figure 2. The relationship between the hemolymph Na^+ concentration and the external Na^+ concentration of juvenile blue crabs not treated with pesticides (o), DDT₁ treated (●), DDT₂ treated (*), and Mirex₃ treated (X) at 25°C and untreated juvenile blue crabs at 16°C (+). The dotted line is from Mantel, 1967 (19-21°C) (adult blue crabs). (Diagonal is equality.)

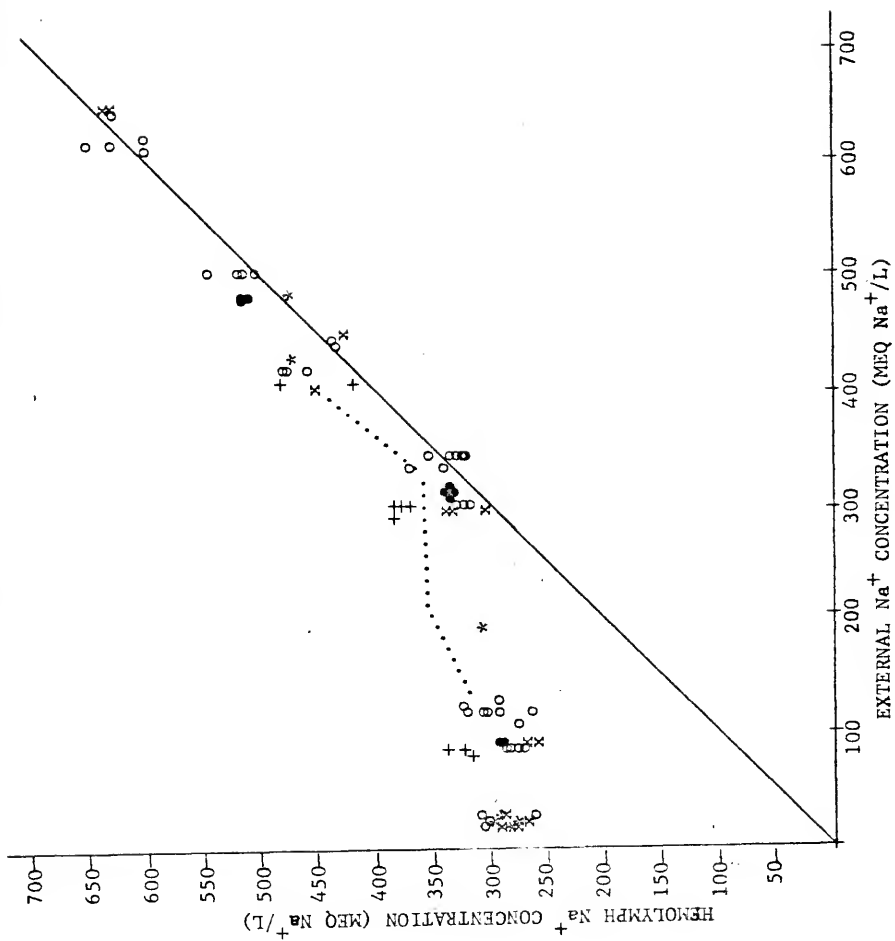
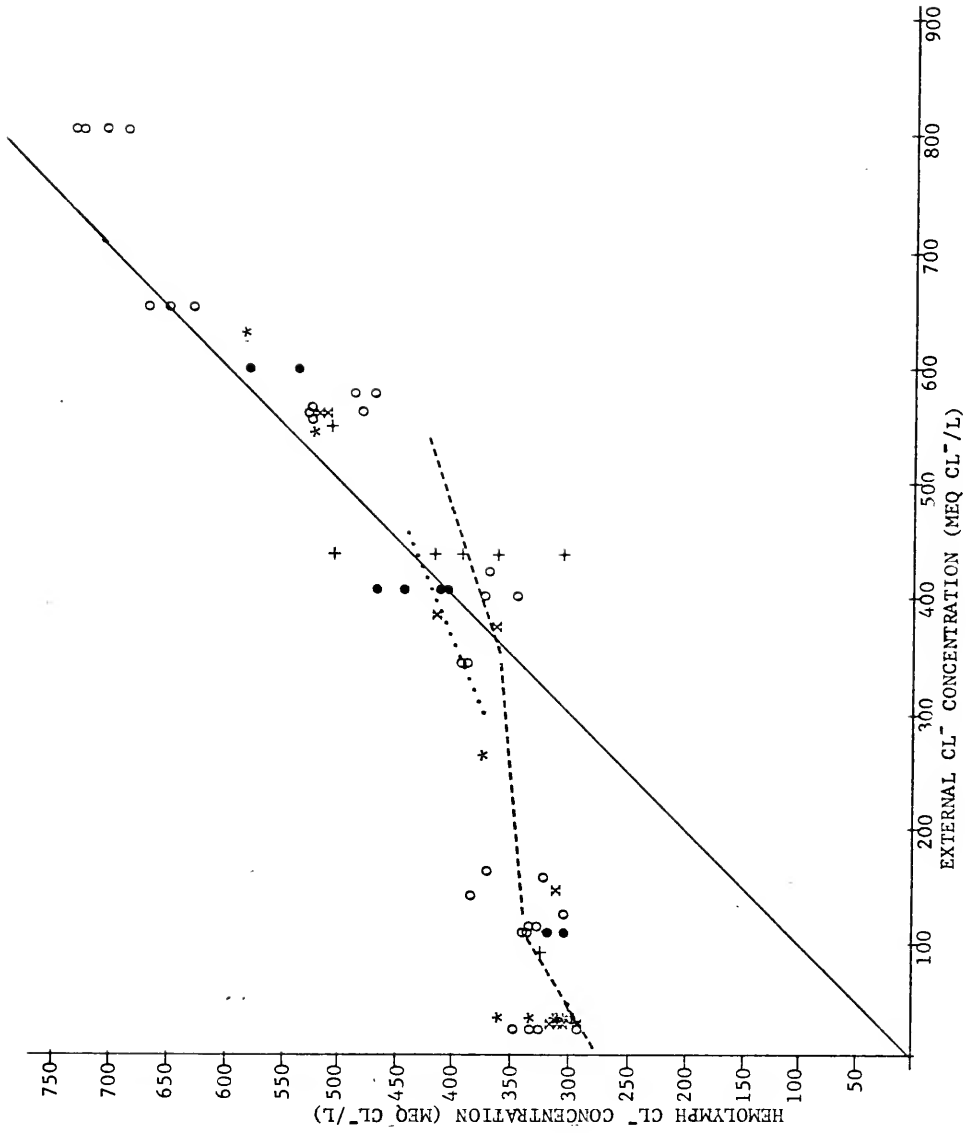


Figure 3. The relationship between the hemolymph Cl^- concentration and the external Cl^- concentration of juvenile blue crabs not treated with pesticides (o), DDT₁ treated (●), DDT₂ treated (*), and Mirex₃ treated (X) at 25°C and untreated juvenile blue crabs at 16°C (+). Lines are for adult blue crabs from Lynch *et al.*, 1973, 25-27°C (-----) and 14-17°C (.....). (Diagonal is equality.)

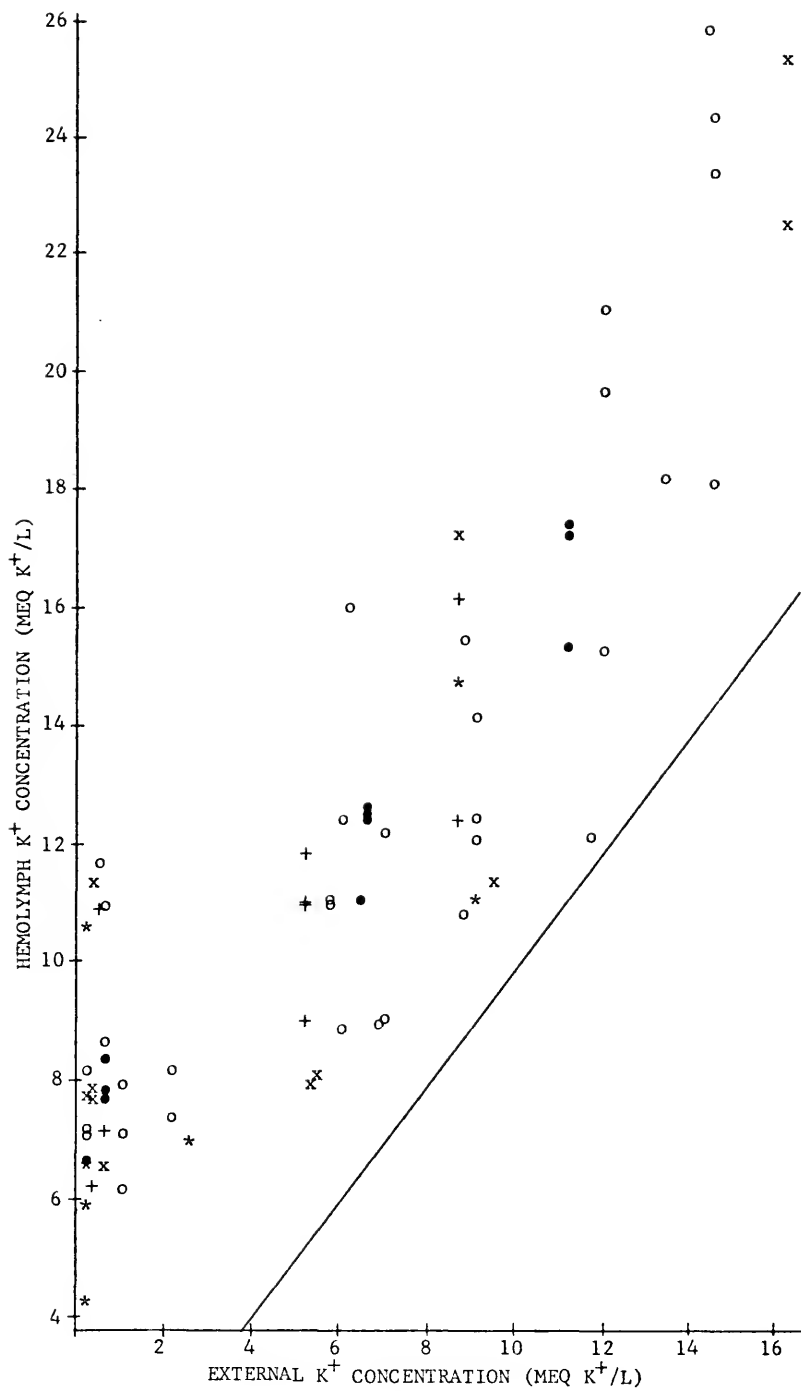


centration is maintained above external concentrations of less than 375 meq/l (660 mOs/l). Within this hyper-regulatory range hemolymph Cl^- is higher at high external concentrations than at low. For example, the hemolymph Cl^- concentration is 325 meq/l when the crab is acclimated to water containing 30 meq Cl^- /l increasing to about 390 meq/l at 350 meq Cl^- /l. Hemolymph Cl^- , unlike Na^+ and total osmotic particles, is hypo-regulated with respect to external concentrations between 400 and 650 meq Cl^- /l (700 and 1200 mOs/l). At an external concentration of 650 meq Cl^- /l the hemolymph Cl^- is isoionic with respect to the medium but at 800 meq Cl^- /l (1410 mOs/l) the hemolymph Cl^- concentration is far below that of the environment.

The hemolymph K^+ concentration is strongly hyper-regulated at all external K^+ concentrations tested (maximum external K^+ concentration = 15 meq/l at 1410 mOs/l).

Temperature also influences the hemolymph concentration of juvenile blue crabs. Below an external concentration 650 mOs/l the hemolymph concentrations of crabs acclimated to 16°C are higher than those of crabs acclimated to 25°C (Figure 1). For example, the mean hemolymph concentration of a crab acclimated to 25°C and 200 mOs/l is about 600 mOs/l but at 16°C and the same external concentration the hemolymph concentration is about 8% higher (647 mOs/l). Similarly, at 25°C and 600 mOs/l, the mean hemolymph concentration is about 700 mOs/l but is about 770 mOs/l at 16°C and the same external concentration. However, at 1000 mOs/l the hemolymph concentrations at 25°C and 16°C were roughly equal.

Figure 4. The relationship between the hemolymph K^+ concentration and the external K^+ concentration of juvenile blue crabs not treated with pesticides (o), DDT₁ treated (●), DDT₂ treated (*), and Mirex₃ treated (X) at 25°C and untreated juvenile blue crabs at 16°C (+). (Diagonal is equality.)



At 16°C, the hemolymph Na^+ concentration is, like total concentration, higher than at 25°C at external Na^+ concentrations less than 400 meq/l (950 mOs/l) (Figure 2). For example, at an external Na^+ concentration of 300 meq/l the hemolymph Na^+ concentration is 375 meq/l when the crab is acclimated to 16°C but only about 325 meq/l at 25°C. The hemolymph K^+ and Cl^- concentrations appear to be unaffected by temperature (16° and 25°C) (Figures 3 and 4).

At 16°C juvenile blue crabs cannot survive exposure to 75 mOs/l if abruptly transferred from 22°C, 800 mOs/l. Of six crabs transferred, none survived more than 12 h.

Salinity Effect on Metabolic Rate

No significant differences in metabolic rate are observed in crabs acclimated to and tested at different salinities between 50 and 1410 mOs/l (25°C) (Figure 5, untreated). However, the metabolic rates of crabs acclimated to one salinity and tested at a lower salinity are much higher at the lower salinity. For example, one juvenile blue crab which had a metabolic rate of 0.221 ml $\text{O}_2/\text{g}\cdot\text{h}$ when acclimated to 456 mOs/l showed an approximately 65% increase in metabolic rate (to 0.36 ml $\text{O}_2/\text{g}\cdot\text{h}$) when transferred to 163 mOs/l. Another crab abruptly transferred from its 1235 mOs/l acclimation medium to 387 mOs/l showed an even greater metabolic rate increase (87%; 0.21 to 0.38 ml $\text{O}_2/\text{g}\cdot\text{h}$) (Figure 5).

Oxygen Concentration Effects on Metabolic Rate

In oxygen rich water, juvenile blue crabs appear to be metabolic regulators. Above 2.0 ml O_2/l , the ambient O_2 concentration did not affect the metabolic rates of juvenile blue crabs at any salinities tested (between 50 and 1410 mOs/l) (Figure 6).

Figure 5. The effect of the environmental concentration on the weight specific O_2 consumption of juvenile blue crabs (25°C, O_2 concentration: 4.5 ml O_2/l) not treated with pesticides (o), DDT₁ treated (●), DDT₂ treated (*), Mirex₁ treated (+) and Mirex_{2, 3} treated (X). T indicates crabs treated with DDT which were exhibiting convulsions. (Ranges indicated are standard errors of means.) The arrows indicate metabolic responses of juvenile blue crabs to sudden changes from higher (origin of arrow) to lower (point of arrow) concentrations.

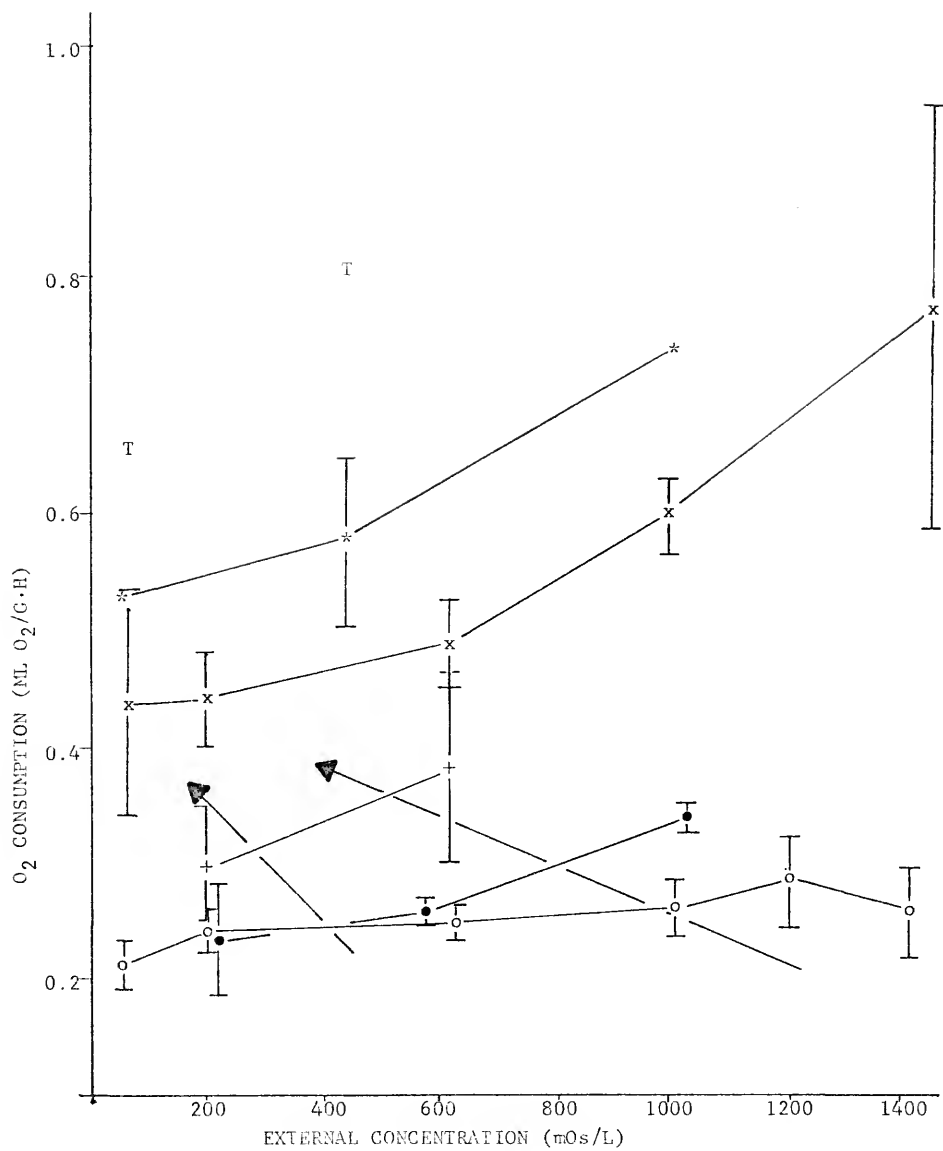
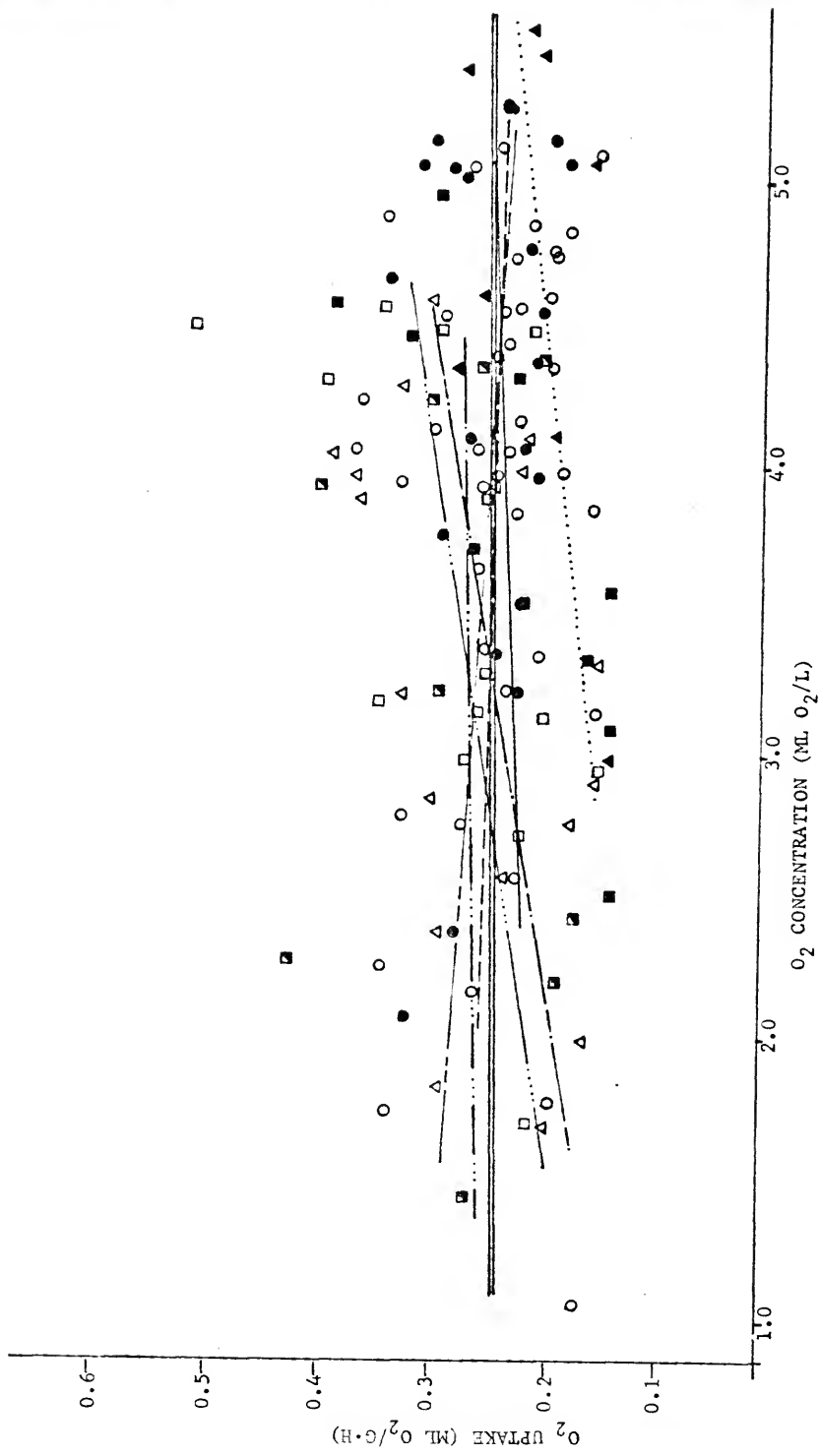


Figure 6. The effect of environmental O_2 concentration on the weight-specific O_2 consumption of untreated juvenile blue crabs (25°C) at various salinities: 50 mOs/l (\blacktriangle ,), 200 mOs/l (\odot , -----), 560 mOs/l (\blacksquare , -----), 610 mOs/l (\circ , -----), 1000 mOs/l (\square ,), 1200 mOs/l (\blacksquare ,), and 1410 mOs/l (\triangle , -----). The heavy double line (=====) is the regression of O_2 uptake on O_2 concentration of all concentrations combined. (Regressions determined by method of least squares.)



Pesticide Concentration in Juvenile Blue Crabs
from the Cedar Key Area

The mean DDT family concentration in juvenile blue crabs collected from the estuarine zone around Cedar Key was 0.031 ± 0.003 ppm. No Mirex was detected in any sample from this area.

Acute Levels of DDT and Mirex

Ingested Mirex is highly toxic to juvenile blue crabs. At intermediate salinities (200-600 mOs/l) four feedings of $0.14 \mu\text{g}$ Mirex each over a five-week period resulted in convulsions in most of the 0.75-1.25 g crabs. Convulsions were induced in 1.25-2.5 g crabs by five such feedings. The internal concentrations of the poisoned crabs were 0.48 ± 0.05 ppm Mirex. In dilute (50 mOs/l) and concentrated (1000 mOs/l) media the crabs were slightly more sensitive to Mirex: three weekly feedings of $0.14 \mu\text{g}$ Mirex each produced acute effects in 0.75-1.25 g crabs and four feedings resulted in convulsions and death in the 1.25-2.5 g crabs: internal concentrations averaged 0.42 ± 0.05 ppm.

DDT is a far less toxic stomach poison to juvenile blue crabs than is Mirex. No acute effects were elicited by DDT at four times the acute Mirex intake levels (four weekly feedings of $0.53 \mu\text{g}$ DDT). Such treatment resulted in DDT family tissue concentrations approximately equal to Mirex tissue concentrations resulting from 1/4 the dietary intake: 0.13 ± 0.01 ppm DDT family, 0.19 ± 0.03 ppm Mirex. Three weekly feedings of $3.2 \mu\text{g}$ DDT resulted in typical symptoms of chlorinated hydrocarbon poisoning at 50 mOs/l and 430 mOs/l in 1.5-2.5 g crabs (internal concentrations: 1.0 ± 0.1 ppm). Crabs killed by DDT (same intake levels as above) at 1000 mOs/l showed none of the characteristic symptoms of chlorinated hydrocarbon

poisoning. The DDT levels in these crabs were 1.1 ± 0.15 ppm. Crabs were treated with high levels of DDT in Mg^{++} enriched (Mg^{++} concentration approximately 1.2 times that of full sea water) brackish water (400 mOs/l) to test the hypothesis that the higher Mg^{++} levels in the sea water might, by relaxing muscles, inhibit the typical chlorinated hydrocarbon syndrome in sea water. These crabs showed typical signs of DDT poisoning at the same input levels as those in non- Mg^{++} enriched brackish water. High Ca^{++} levels were tried in an attempt to raise the threshold of nerves and thus suppress the symptoms. The results were as with Mg^{++} . Mirex treated crabs at high salinities (1000 and 1450 mOs/l) showed typical symptoms of chlorinated hydrocarbon poisoning.

Pesticide Effects on Metabolic Rate

High subacute internal levels of Mirex and DDT (0.19 ± 0.03 ppm Mirex = Mirex₃, 0.82 ± 0.05 ppm DDT = DDT₂*) cause pronounced elevations of juvenile blue crab metabolic rates (Figure 5). The metabolic rates of these pesticide treated crabs are more than twice those of untreated crabs. Some subtle behavioral abnormalities such as greater than normal reactions to food, extreme excitability when pursued, and a tendency to move in a "tip-toe" manner were observed at these subacute pesticide levels. Tissue Mirex levels causing metabolic rate increases are much lower than the internal DDT family concentrations that elevate the metabolic rate: DDT family concentrations (0.15 ppm = DDT₁) approximately equal to the Mirex

*See Table 1.

levels (0.19 ppm) that result in metabolic rates double those of untreated crabs have no effect on the metabolic rates of the crabs (200 and 600 mOs/l) (Figure 5). Mirex concentrations (Mirex₁) as low as 0.02 parts per million parts of body tissue caused significant metabolic rate elevations (Figure 5). Although the differences are not significant at the 95% confidence level (Student-t), the pesticide caused metabolic rate elevation is more pronounced at higher salinities (Figure 5). For example, the mean metabolic rate of Mirex₃ treated crabs at 1000 mOs/l is 1.25 times those at 50 or 200 mOs/l. Mirex₃ crabs at 1450 mOs/l had metabolic rates averaging 1.8 higher than at 50 mOs/l. Similar increases were observed with DDT treatments. DDT₁ crabs had the same rates of metabolism as controls at 200 and 600 mOs/l but the same treatment caused significant metabolic rate increases at 1000 mOs/l (Figure 5).

Oxygen Concentration Effects on the Metabolic Rates of Pesticide Treated Juvenile Blue Crabs

Metabolic rates of crabs fed pesticides are independent of environmental O₂ concentration at high O₂ levels (Figures 7, 8, and 9). Crabs that were exposed to O₂ concentrations of less than 2.0-2.5 ml O₂/l consumed O₂ more slowly (Figures 7, 8, and 9). Although the sample size is too small to draw a definite conclusion, Mirex treated crabs at higher salinities (1000 and 1450 mOs/l) appear more sensitive to falling O₂ concentrations than crabs in other groups (*i.e.* the critical oxygen concentration may be as high as 2.5 ml O₂/l) (Figure 9).

Figure 7.

The effect of environmental O_2 concentration on the weight-specific O_2 consumption of DDT treated juvenile blue crabs (25°C) at various salinities: DDT₁, 210 mOs/l (O, -----), DDT₁, 570 mOs/l (C, --- -- ---), DDT₁, 1030 mOs/l (□, -----), DDT₂, 50 mOs/l (◇,), DDT₂, 430 mOs/l (△), and DDT₂, 1000 mOs/l (△, -----).

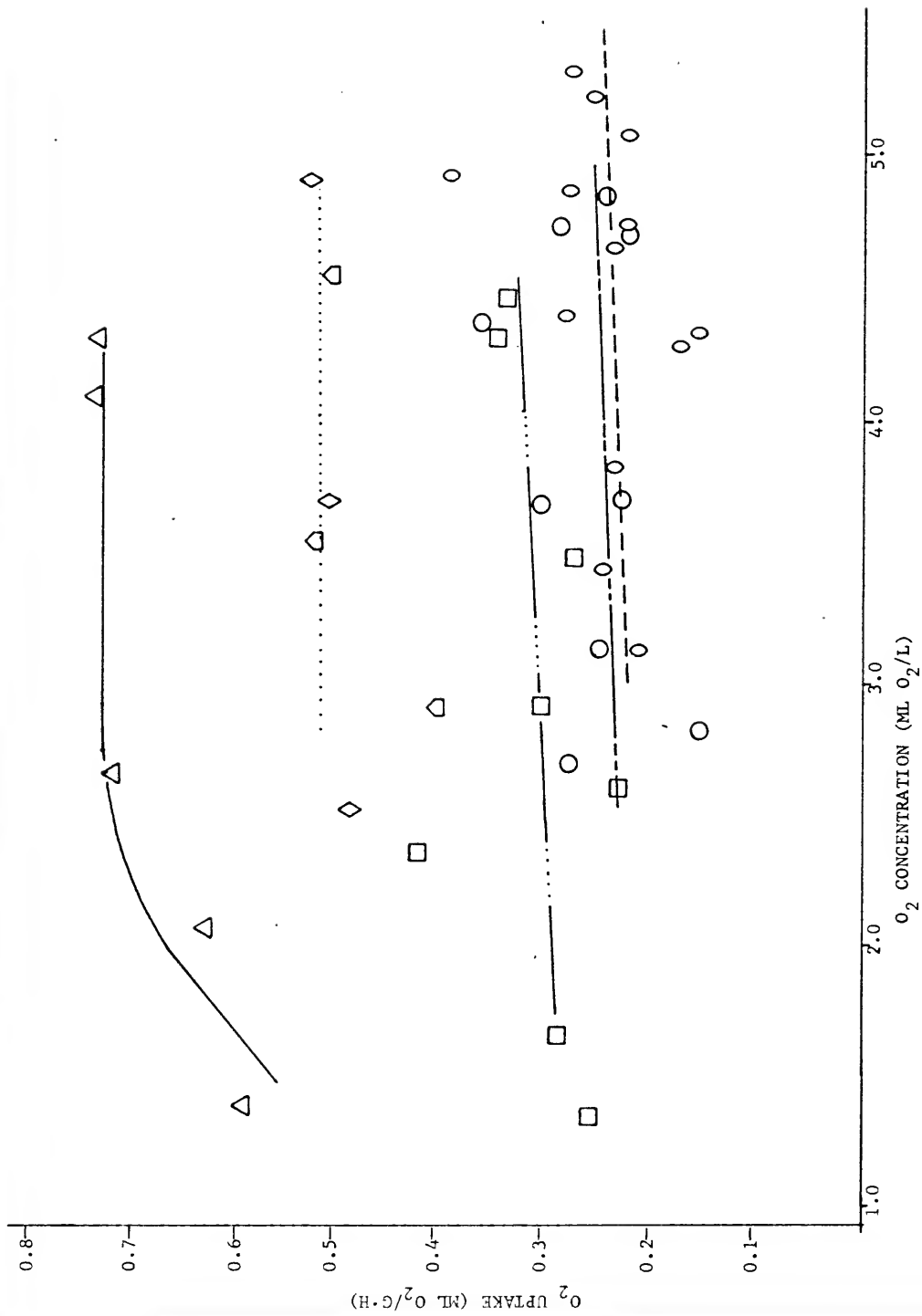


Figure 8. The effect of environmental O₂ concentration on the weight-specific O₂ consumption of Mirex treated juvenile blue crabs (25°C) at various salinities: Mirex₁, 200 mOs/l (Δ, ———), Mirex₂, 200 mOs/l (○, -----), Mirex₃, 50 mOs/l (◇, !....), and Mirex₃, 200 mOs/l (◊).

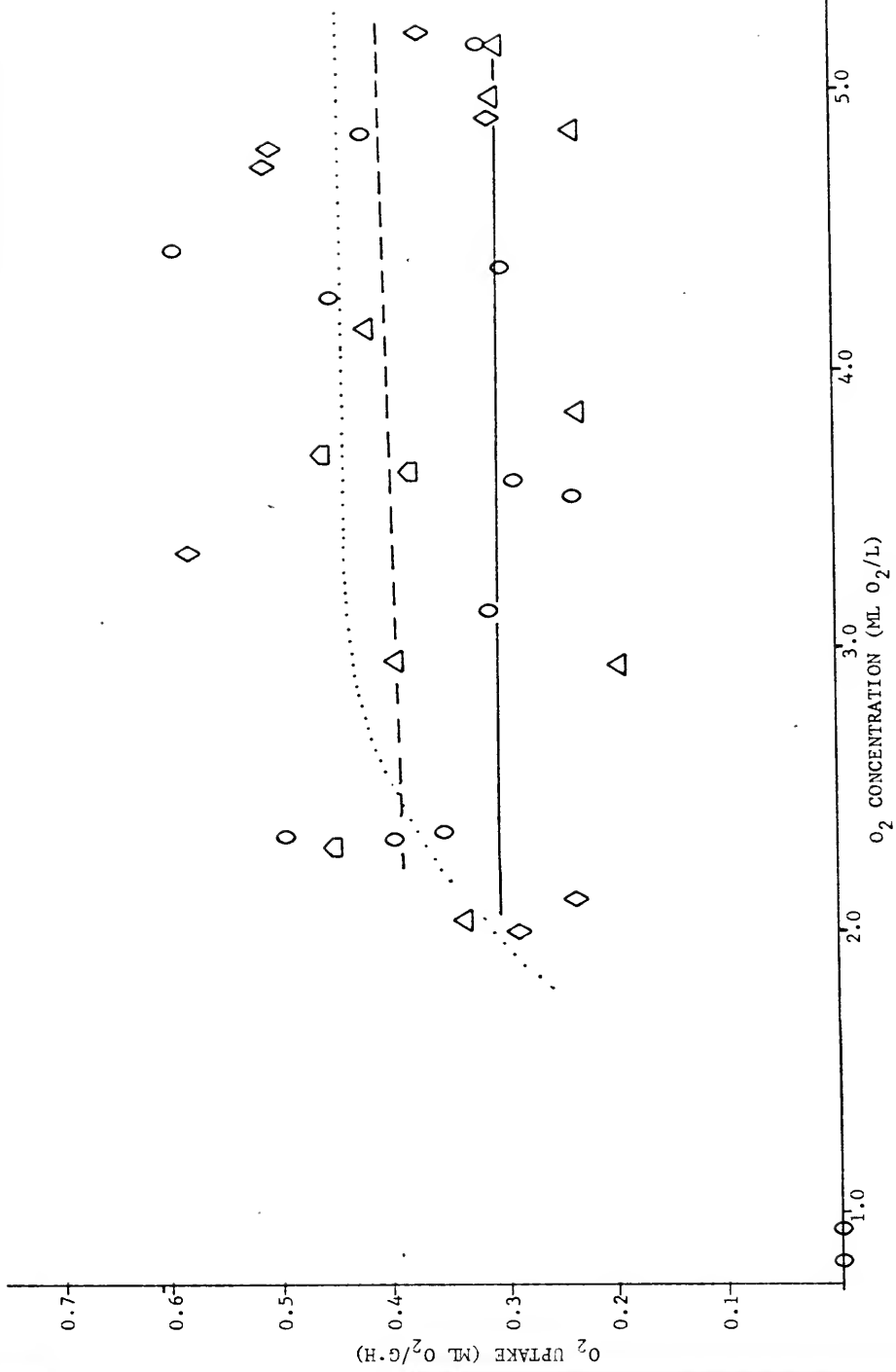
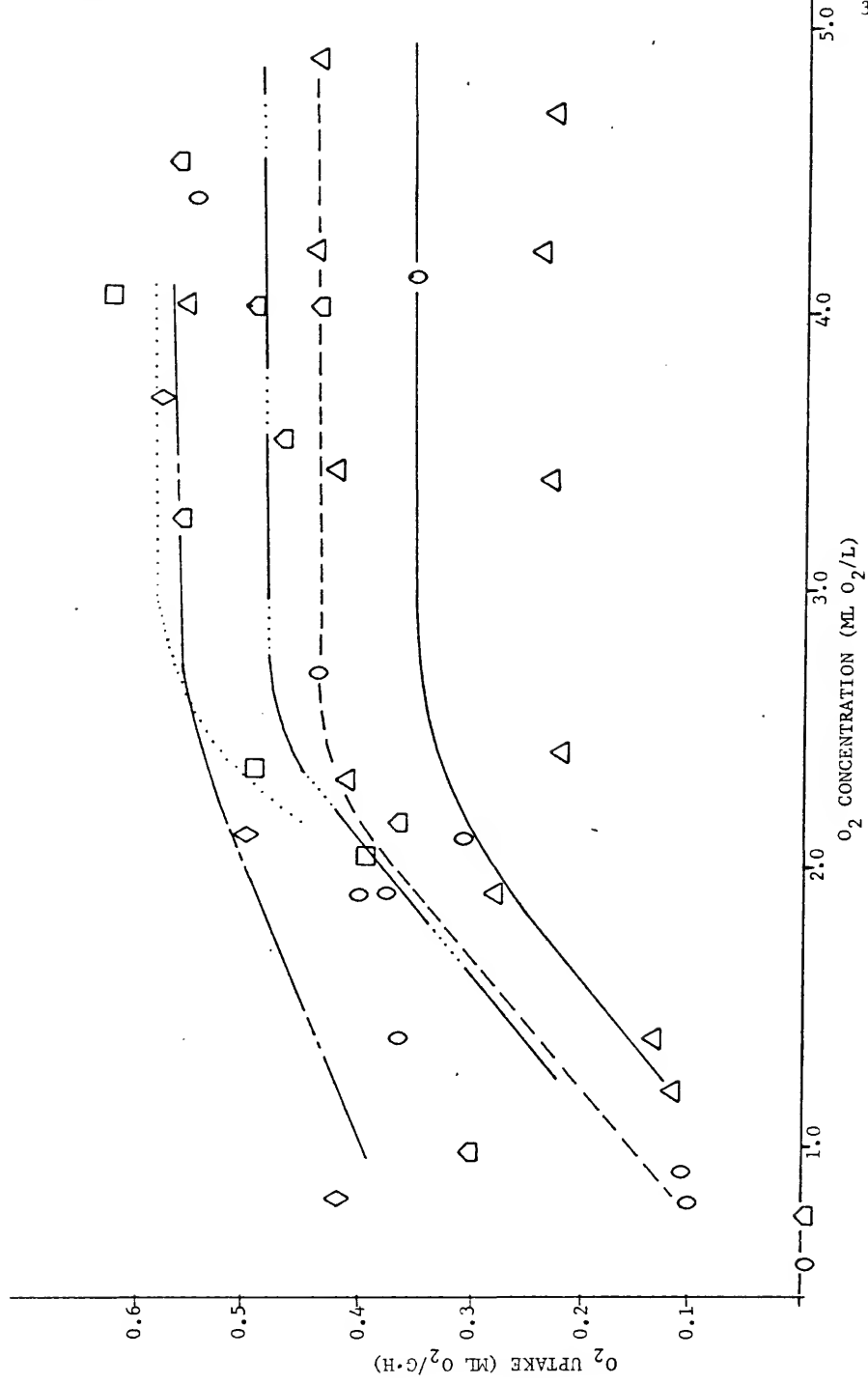


Figure 9. The effect of environmental O₂ concentration on the weight-specific O₂ consumption of Mirex treated juvenile blue crabs (25°C) at various salinities: Mirex₁, 610 mOs/l (△, ———), Mirex₂, 610 mOs/l (○, ----), Mirex₃, 610 mOs/l (◇,), Mirex₃, 1000 mOs/l (□,), and Mirex₃, 1450 mOs/l (◇, --- - - ---).



Pesticide Effects on Ionic and Osmotic Regulation

DDT and Mirex do not significantly affect osmotic and ionic regulatory patterns of juvenile blue crabs at subacute concentrations (Figures 1, 2, 3, and 4).

Pesticide Effects on Autotomization of Limbs

High levels of Mirex inhibit the ability of juvenile blue crabs to autotomize limbs. The inhibition applies to both the autotomy of a single damaged limb (Figure 10) and to the number of limbs autotomized in response to extreme generalized stimuli (Figure 11). Internal Mirex concentrations as low as 0.02 ppm (Mirex₁) inhibited autotomy of limbs by crabs placed in hot water (one limb autotomized per crab compared to an average of more than 3 limbs per untreated crabs). The same Mirex concentration blocks autotomy of a severely damaged limb by most crabs. Ninety-five percent of the untreated crabs autotomized a walking leg with a crushed merus while only 29% of Mirex treated crabs autotomized a limb with the same damage (Figure 10, results from all crabs with Mirex levels between 0.02 and 0.2 ppm are combined as no concentration-dependent response was observed). Extremely high internal DDT concentrations (0.82 ppm [DDT₂]), although causing some inhibition (less than 2 limbs per crab compared to an average of more than 3 limbs per untreated crab) of limb autotomy in response to hot water (Figure 11), did not inhibit autotomy of a damaged limb (Figure 10). Juvenile blue crabs exhibiting convulsions resulting from either DDT or Mirex poisoning were unable to autotomize limbs. Seven convulsive animals (4 Mirex induced, 3 DDT induced) were tested for ability to autotomize a damaged walking leg

Figure 10. The effect of pesticide treatment (Mirex₁, 2, 3, DDT₁, DDT₂) on the ability of juvenile blue crabs to autotomize a damaged walking leg. (Numerals within the bars indicate sample size.)

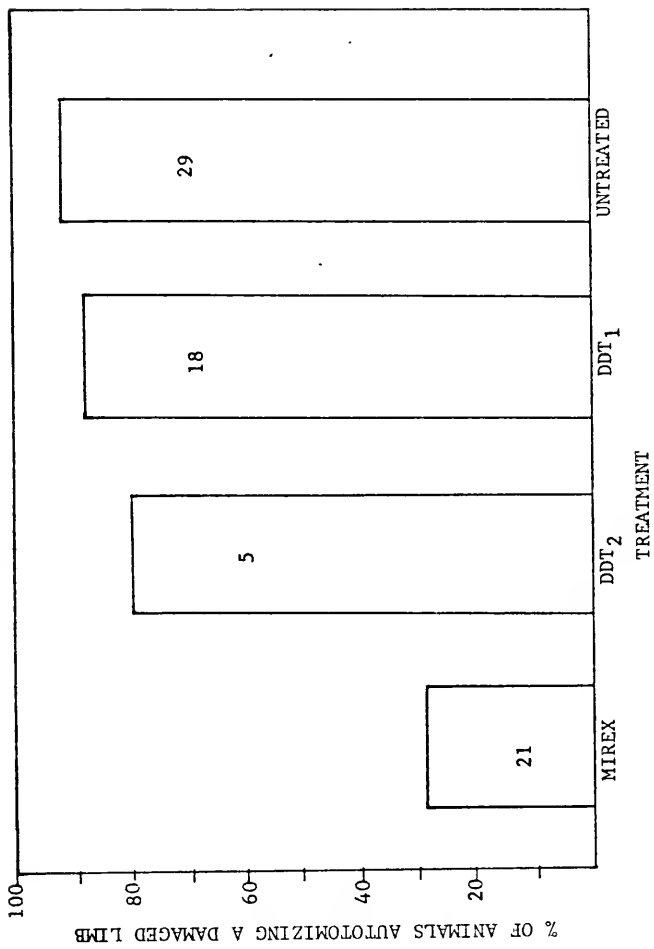
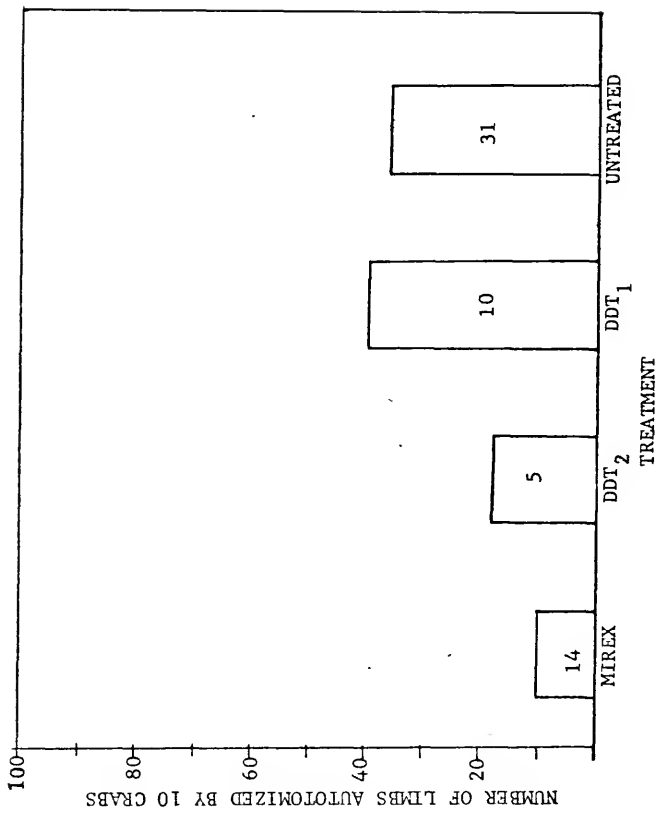


Figure 11. The effect of pesticide treatment (Mirex₁, 2, 3, DDT₁, DDT₂) on the number of limbs autotomized by juvenile blue crabs when placed in hot (60°C) water. (Numerals within the bars indicate sample sizes.)



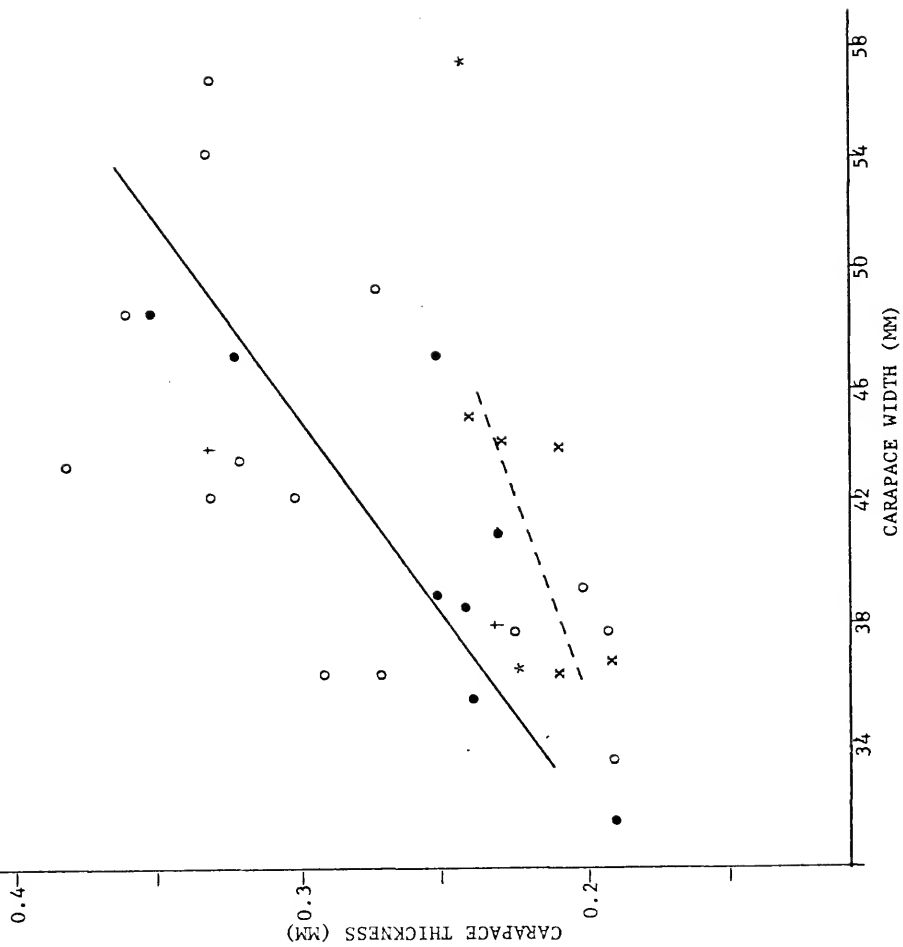
or to autotomize limbs when placed in hot water. One chela was autotomized by a Mirex treated crab in hot water--no other autotomy occurred.

Pesticide Effects on Carapace Thickness

When I measured the carapace thicknesses and widths of crabs molting during captivity (during molt cycle stage C_4), I discovered that the thicknesses compared to the widths of Mirex₃ treated crabs were considerably less than those of the controls (Figure 12). For example, an average 42 mm wide untreated juvenile blue crab has a carapace that is 0.27 mm thick while an average 42 mm wide Mirex₃ juvenile blue crab has a carapace that is only 0.22 mm thick mid-dorsally. Crabs treated with lesser amounts of Mirex (Mirex₂) and DDT₁ did not have unusually thin carapaces. The data on carapace thicknesses of DDT₂ crabs are sparse. However, these data are similar to those from Mirex₃ crabs (Figure 12).

Figure 12.

The carapace thicknesses relative to widths in C_d of juvenile blue crabs fed untreated food (o, -----), DDT₁ (●), DDT₂ (*), Mirex₂ (+), and Mirex₃ (X, -----). (Regressions were determined by method of least squares.)



DISCUSSION

Hemolymph osmotic, Na^+ , and Cl^- concentrations in adult blue crabs have been extensively studied at external concentrations of up to 1000 mOs/l. No significant differences in regulatory patterns of juveniles and adults are observed (Figures 1, 2, and 3). Gifford (1962) and Ballard and Abbott (1969) have measured adult hemolymph concentrations at very high salinities. Their results differ (Figure 1). Gifford (1962) reports hypo-osmoregulation at all salinities above 1100 mOs/l while Ballard and Abbott (1969) report slight hypo-osmoregulation between 900 and 1100 mOs/l and osmoconformity above these salinities. My results on juveniles are intermediate with respect to the conclusions of these authors. I detected very slight hypo-osmoregulation above 1100 mOs/l (25°C) (Figure 1) due apparently to Cl^- hypo-regulation. Chloride hypo-regulation in juveniles is similar to that of total hemolymph concentration reported by Ballard and Abbott (1969) between 900 and 1100 mOs/l. However, Cl^- is also strongly hypo-regulated at 800 meq/l (1410 mOs/l) (Figure 3). Lynch *et al.* (1973) observed hypo-regulation of Cl^- in adults between external concentrations of 400 and 550 meq Cl^- /l. Chloride and Na^+ regulatory patterns are noticeably different which is what one would expect if these ions are independently transported as suggested by Mantel (1967).

Temperature affects osmoregulation in juvenile blue crabs. Numerous investigators have noted that adult blue crabs have higher hemolymph

salt concentrations in cold water than in warmer water of the same salinity (for example, Lynch *et al.*, 1973). This phenomenon was also observed in juvenile blue crabs acclimated to 25° and 16°C at salinities less than 700 mOs/l (Figure 1). Part of the hemolymph concentration increase in cool water is due to a hemolymph Na⁺ concentration increase (Figure 2). Chloride and K⁺ are not affected by temperature (Figures 3 and 4). The inability of juvenile blue crabs to tolerate low temperature (16°C)/low salinity (76 mOs/l) combinations is important. This limitation may explain the observation that in temperate regions small crabs migrating into the estuarine zone in the fall after completing their larval development do not migrate into fresh water bays and rivers until the following spring (Van Engel, 1958).

There were no significant differences between metabolic rates of crabs acclimated to different salinities between 50 and 1410 mOs/l (Figure 5). Such absence of metabolic rate variation with salinity is expected in an animal that does not strongly hypo-osmoregulate. Hyper-osmoregulation does not appear to be an energetically expensive function. Potts (1954) proposed a minimal theoretical energy expenditure for osmotic regulation by *Eriocheir* in hard, fresh water as low as 0.5% of the standard metabolic rate of a 60 g (wet) crab. This value is based on the assumptions that all ion loss is via urine and that active transport is 100% efficient. Seventy-five percent of the Cl⁻ loss by *Eriocheir* is at the body surface Shaw (1961). Most transport processes are between 20 and 80% efficient (Potts and Parry, 1964). Taking these factors into account, the expected energetic cost of osmotic regulation

by a 60 g *Eriocheir* in hard, fresh water is between 2.5 and 10% of its standard metabolic rate. My results, as well as earlier experiments on an euryhaline fish (*Mugil cephalus*; Nordlie and Leffler, 1974), support the prediction that the steady-state energetic cost of hyper-osmoregulation may be very low. Metabolic rates of crabs acclimated to the low test salinity and those acclimated to salinities higher than the test salinity may be greatly different. While juvenile blue crabs acclimated to the test salinities have similar metabolic rates at 50, 200, 600, 1000, 1200, and 1410 mOs/l, crabs abruptly transferred from higher salinities to lower ones have greatly elevated metabolic rates. The fact that the steady-state and transitory relationships between metabolic rate and salinity are so dissimilar indicates that increases in metabolic rate following transfer from one medium to a more dilute one are not totally the result of increases in the metabolic rates of the salt absorbing tissues per se, but, also, the result of metabolic rate increases in tissues not directly associated with osmoregulation. Further supporting this contention, the magnitude of the difference between acclimation salinity and test salinity is apparently more important than the level of the test salinity in determining the extent of the increase. For example, a crab acclimated to 1200 mOs/l had a metabolic rate 87% higher than expected at 450 mOs/l but a crab acclimated to 450 mOs/l and tested at 160 mOs/l had a metabolic rate only 65% higher than expected (Figure 5). No pronounced increase in metabolic rate was expected at high salinities because juvenile blue crabs do not strongly hypo-osmoregulate. The absence of observable metabolic rate differences between crabs acclimated to different salinities confirmed this expectation (Figure 5).

Juvenile blue crabs are nonburrowing inhabitants of open portions of the estuarine zone. They are not frequently exposed to low O_2 concentrations. These crabs are metabolic regulators in the high environmental O_2 concentration range to which they are commonly exposed. The mean standard metabolic rates of juvenile blue crabs are the same at 2.0 and 5.5* ml O_2/l (Figure 6). Earlier measurements of juvenile blue crabs' metabolic rates at lower O_2 concentrations indicated that these crabs were metabolic conformers (Leffler, 1972). The critical oxygen pressure cannot be pinpointed because of differences between the methods used. It is below 2.0 ml O_2/l . The hyperbolic curve of metabolic rate on O_2 concentration is much different from the linear relationship (with a very gradual slope) observed in the burrowing Xanthid crabs, *Menippe mercenaria* and *Panopeus herbstii* (Leffler, 1973), that naturally encounter low O_2 concentrations.

Chlorinated hydrocarbons are ubiquitous in the environment. DDT has been applied globally since 1946. The application of another chlorinated hydrocarbon, Mirex, has, thus far, been more restricted. Since 1963 it has been applied to vast areas of southeastern U.S.A. in an attempt to control the imported fire ant, *Solenopsis invicta* (Bellinger *et al.*, 1964; Coon and Fleet, 1970; Collins *et al.*, 1973).

Aquatic, estuarine, and marine carnivores, scavengers, and detritus feeders take up chlorinated hydrocarbons mainly via their food. Chlorinated hydrocarbons have low solubilities in water. They adhere to various organic particles allowing input into detritus feeders to be high (Odum *et al.*, 1969). Biological magnification of persistent pesticides

*5.5 ml O_2/l was the highest O_2 concentration at which experiments were performed. Saturation at 25°C, 50 mOs/l is 5.7 ml O_2/l .

in estuarine food chains has been demonstrated (Woodwell *et al.*, 1967). Therefore, the food of scavengers and predators would contain DDT family and Mirex concentrations far above those in the water. Macek and Korn (1970) support this contention. Their work shows that brook trout accumulate DDT at a ratio of 10 parts from food to each part taken up directly from the water. Catfish denied access to their natural food chain in Mirex treated ponds did not accumulate Mirex while free-living catfish in the same ponds contained 0.65 ppm after 6 months (Collins *et al.*, 1973). Pesticide input into a detritus feeder, scavenger, and active predator such as *Callinectes* would also be mainly via its food. In addition, juvenile blue crabs have been shown to ingest Mirex bait with fatal results when such bait is accessible (Mahood *et al.*, 1970).

With food being the main route of chlorinated hydrocarbon input into many aquatic, estuarine, and marine animals, it is surprising to find that nearly all laboratory studies of the effects of persistent pesticides on these animals have been conducted by pesticide application to the aqueous medium. This approach can produce misleading results. Stomach poisons (*i.e.* input through the digestive system) may be relatively nontoxic contact poisons while potent contact poisons may be relatively nontoxic stomach poisons. Butler (1963) states that Mirex in solution is relatively nontoxic to juvenile blue crabs (48 h, $EC_{20} = 2$ ppm [compared to DDT with a 48 h $EC_{50} = 0.01$ ppm]). In contrast, ingested Mirex has far greater effects on juvenile blue crabs than does ingested DDT. Acute Mirex intake levels are about 0.06 those of DDT. However, the internal DDT family concentrations of juvenile blue crabs

fed amounts of DDT 17 times greater than the amounts of Mirex fed to other crabs were only twice the Mirex levels in the latter group. Either (1) the digestive system absorbs Mirex more readily than it does DDT, or (2) DDT and its metabolites are excreted at greater rates than Mirex. The second alternative must be at least in part responsible for the lower retention of ingested DDT compared to Mirex because several DDT poisoned animals exhibiting mild chlorinated hydrocarbon syndrome recovered while no Mirex treated crab showing signs of poisoning ever recovered. Mirex is also more potent than is DDT in eliciting subacute responses (*i.e.* increased metabolic rate, inhibition of autotomy reflex). Mirex produces pronounced metabolic rate increases at levels less than 0.25 internal DDT family concentrations resulting in similar responses.

Acute and subacute DDT family and Mirex concentrations being discussed are not above those encountered in many estuarine food chains. For example, the maximum Mirex levels that Mahood *et al.* (1970) detected in the tissues of adult blue crabs from Georgia coasts was 0.389 ppm. A juvenile blue crab (1.25 g) ingesting 1.44 g of such a carcass in 4 weeks would receive a lethal Mirex dose. Mahood *et al.* (1970) measured levels of DDT and its metabolites as high as 0.231 ppm in adult blue crabs from Georgia. Forty grams of this tissue eaten in 4 weeks would be acute to 1 g crabs. Other prospective food items have pesticide concentrations far in excess of these: (Long Island, New York, U.S.A.) white perch, 1.99 ppm DDT family; menhaden, 1.53 ppm DDT family (Foehrenbach, 1972); sheepshead minnow, 0.94 ppm DDT family; chain pickerel, 1.33 ppm DDT family; Atlantic

needle fish, 2.07 ppm DDT family (Woodwell *et al.*, 1967). Even the vegetation may contain high pesticide levels. Vegetation in ponds treated with Mirex bait at the manufacturer's recommended application rate for field use contained Mirex residues as high as 4.0 ppm (Van Valin *et al.*, 1968). Juvenile blue crabs devour more than 55% of their wet weights in food per day (Holland *et al.*, 1971). I found the wet weight/dry weight ratios were greater than 5. Therefore, disregarding growth, a 1 g blue crab would consume at least 70 g of food in 4 weeks.

High, subacute concentrations of DDT and Mirex result in pronounced elevations of juvenile blue crab standard metabolic rates (Figure 5). This elevation was observed at all concentrations above the minimal level eliciting the response. Even crabs exhibiting pesticide induced convulsions had as high metabolic rates as crabs with high subacute concentrations. This is in contrast with *Physa gyrina* (pond snail), *Gambusia affinis* (mosquitofish), and *Lepomis macrochirus* (sunfish) in which low Mirex concentrations result in metabolic rate elevations while higher concentrations depress rates of metabolism (De La Cruz and Naqvi, 1973). I believe the metabolic rate increases are the result of increased muscle and nerve metabolism. DDT (and presumably other chlorinated hydrocarbons) has an unstabilizing effect on nerves and muscles (Yeager and Munson, 1945; Roeder and Weiant, 1945, 1948; Bodenstein, 1946; Welsh and Gordon, 1947). Increased muscular and nervous activity must increase the metabolic rates of these tissues substantially. In addition, DDT delays the turning off of the Na^+ influx associated with action potential development (Harahashi and Haas, 1968). The resulting overlap might

increase the necessary energy expenditure associated with returning the nerve to its "resting" state following a discharge.

Juvenile blue crabs with pesticide induced metabolic rate elevations might face difficulties in obtaining sufficient food and oxygen to accommodate both normal growth and increased metabolism. Blue crabs with elevated metabolic rates as juveniles caused by high ambient temperatures are smaller at maturity than are crabs that grow up in cooler water (Leffler, 1972). Increased metabolic rates of juvenile blue crabs containing substantial subacute chlorinated hydrocarbon concentrations might similarly result in a reduced molt increment and, thus, reduced size at the terminal molt. Reduced adult size, if it did result from high internal pesticide concentrations, as well as increased resting energy expenditure might adversely affect reproductive success. In hypoxic waters crabs with high metabolic rates face insufficient O_2 at higher concentrations than those that limit crabs with lower metabolic rates. Thus, chlorinated hydrocarbons may act synergistically with other conditions in making particular waters unsuitable for blue crab populations.

Mirex concentrations far below acute levels inhibit the autotomy reflex of juvenile blue crabs (Figures 10 and 11). It may be that this inhibition is associated with a reduction of internal acetylcholine. High acetylcholine levels (via injection) facilitate autotomy in crustaceans while compounds (*e.g.* atropine) that prevent normal acetylcholine action result in partial or complete inhibition of the reflex (Welsh and Haskin, 1939). High DDT levels reduce acetylcholine concentrations in the cerebral cortex and striatum of rats (Hrdina *et al.*, 1973). Rosenblueth

and Morison (1937) suggest that high frequency stimulation of vertebrate nerves reduces the quantal yield of acetylcholine per impulse. Such a mechanism has been proposed for the inhibition of the autotomy reflex as more legs are autotomized (Welsh and Haskin, 1939). Such increased nervous activity is caused by chlorinated hydrocarbons. Whatever the cause of the inhibition of autotomy may be, Mirex is more effective than DDT in producing the altered internal conditions which result in this inhibition. In fact, DDT does not elicit the response until acute levels are reached.

Chlorinated hydrocarbons tend to reduce the thicknesses of completed (Stage C₄) juvenile blue crab carapaces (Figure 12). Persistent pesticides have been implicated in thinning or reduction in total bulk of other calcified tissues. Several chlorinated hydrocarbons when ingested by female birds result in a reduction of the shell thicknesses of their eggs (Peakall, 1970). Immature Roman snails fed small amounts of DDT grow thinner shells than controls (Cooke and Pollard, 1973). Chlorinated hydrocarbons can reduce oyster shell growth (Butler, 1966). Extensive investigations have been and are being carried out into the mechanisms of pesticide related bird egg shell thinning. Apparently, no single mechanism is sufficient to explain this phenomenon. The thinning appears partly associated with enzyme inhibition and affected enzyme production in complex integrated enzyme and hormonal systems associated with egg laying (Peakall, 1970; Cooke, 1973). Similarly, crustacean molting and carapace formation is a complex process involving numerous enzymatic reactions mediated by neurosecretions and hormones (Passano, 1960). Thus, probably no single mechanism is responsible for the

reduction in carapace thicknesses of juvenile blue crabs containing appreciable Mirex and DDT concentrations from Stage E (exuviation) until C₄ ("intermolt").

Since the mid 1960's the blue crab industry along the coasts of the southeastern U.S.A. has experienced a sharp decline in production. Extensive adult mortality has been observed in some areas. However, such crab kills are not sufficient to explain the nearly 50% drop in production between 1964 and 1968 (Mahood *et al.*, 1970). My results raise the possibility that pesticide contamination of estuarine food chains might result in a reduction in juvenile blue crab populations and a subsequent reduction in adult populations. It is not surprising that juvenile blue crab kills are not observed. The estuarine environment abounds with scavengers and predators. Carcasses are only recovered when large animals die and wash ashore or when massive mortality of huge populations occurs--as, for example, red tide caused fish kills. Further, many detrimental effects of chlorinated hydrocarbons on juvenile blue crabs would reduce the likelihood of survival to maturity without mortality directly attributable to acute toxicity. For example, increased excitability and lack of maximal muscular coordination, both components of chlorinated hydrocarbon syndrome, undoubtedly increase susceptibility to predators. DDT induced hyperactivity in frog tadpoles greatly increases their vulnerability to predation by newts (Cooke, 1971). The thinner carapaces characteristic of crabs depending on chlorinated hydrocarbon contaminated food chains might result in greater probability of attacks upon such crabs being successful. Ability to autotomize limbs is important to young crabs.

Inhibition of this ability must have profound effects upon the probability of successfully transversing the juvenile period. Often a limb grasped by a predator will be autotomized and the crab will escape. A damaged appendage is a handicap. Openings in the carapace at the injury site as well as dying or dead tissue increase the likelihood of infection. A damaged leg hampers effective locomotion and hinders both predatory ability and ability to escape predation. A damaged limb as well may impede successful exuviation as the damaged tissue may not easily slip out of the old carapace. Finally, inability to autotomize damaged appendages results in an inability to regenerate new ones. Until the old limb is removed a limb bud cannot form and the useless leg cannot be replaced by a functional one.

The possibilities of ingested chlorinated hydrocarbon pesticides resulting in outright poisoning, metabolic rate elevations, decreased muscular coordination, inhibition of the autotomy reflex, and reduced carapace thickness/width ratio leave little doubt that chlorinated hydrocarbon compounds (specifically DDT and Mirex) are potentially disastrous agents with respect to blue crab populations. Additionally, Mirex, at least with respect to crustaceans (see also Lowe *et al.*, 1971), is far more hazardous than is DDT.

SUMMARY

Experiments were conducted to (1) observe the regulation of hemolymph Na^+ , K^+ , Cl^- , and total osmotic concentration by juvenile blue crabs, (2) measure metabolic costs of osmotic regulation by juvenile blue crabs at different salinities, (3) discover DDT and Mirex effects on metabolic rate, osmotic and ionic regulation, ability to autotomize damaged limbs, and carapace thicknesses of juvenile blue crabs.

1) Juvenile blue crabs at 25°C hyper-osmoregulate when in media less than 700 mOs/l and osmoconform at higher concentrations.

2) Hemolymph Na^+ and Cl^- concentrations gradually increase with increasing external concentrations below 700 mOs/l and increase more rapidly at higher concentrations. Chloride is hypo-regulated between ambient concentrations of 400 and 600 meq Cl^- /l and above 700 meq Cl^- /l.

3) The hemolymph K^+ concentration is higher than the external K^+ concentration at all external concentrations tested (as high as 15 meq/l) (1410 mOs/l).

4) At 16°C and external concentrations less than 700 mOs/l the total osmotic concentration and Na^+ concentration of the hemolymph are higher than at 25°C. Hemolymph Cl^- and K^+ concentrations are the same at 16°C as they are at 25°C.

5) The metabolic rates of juvenile blue crabs acclimated to test concentrations between 50 to 1410 mOs/l are not different. Abrupt

transfer from a higher acclimation salinity to a lower test salinity results in a large increase in the metabolic rate.

6) At O_2 concentrations greater than 2.0 ml O_2 /l juvenile blue crabs are metabolic regulators. This finding in conjunction with my earlier work suggests that the metabolic response of juvenile blue crabs to O_2 concentration results in a hyperbolic curve.

7) The mean DDT concentration in juvenile blue crabs from the Cedar Key, Florida, U.S.A., estuarine zone was 0.031 ppm. No Mirex was detected in any sample from this area.

8) Juvenile blue crabs are sensitive to ingested Mirex and DDT. Mirex is a much more potent stomach poison than is DDT.

9) High, subacute internal levels of Mirex (0.02-0.2 ppm) and DDT (0.8 ppm) cause pronounced metabolic rate elevations. Possible mechanisms behind this phenomenon are discussed.

10) The critical oxygen concentration is higher in crabs with high internal levels of DDT and Mirex than in untreated crabs.

11) Subacute levels of DDT and Mirex do not affect osmotic and ionic regulation by juvenile blue crabs.

12) Mirex internal concentrations 0.02 ppm and above inhibit the ability of juvenile blue crabs to autotomize limbs. Possible mechanisms behind this phenomenon are discussed.

13) High internal levels of Mirex during carapace formation following a molt seem to result in decreases in carapace thicknesses relative to carapace widths.

14) The probable impact of Mirex and DDT contamination of estuarine food chains is discussed. I conclude that DDT and, to a far greater

extent, Mirex are potentially disastrous agents with respect to blue crab populations.

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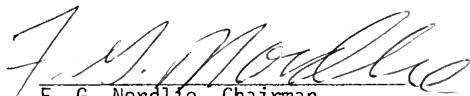
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BIOGRAPHICAL SKETCH

Charles William Leffler, II was born May 21, 1947, at Cleveland, Ohio. In June, 1965, he graduated from Marion Harding High School, Marion, Ohio. From 1965 until 1968, he attended DePauw University, Greencastle, Indiana. In June, 1969, he received the degree of Bachelor of Science with a major in biology from the University of Miami, Coral Gables, Florida. In 1969, he enrolled in the Graduate School of the University of Florida where he received the degree of Master of Science with a major in zoology in 1971. Since that time he has worked toward the degree of Doctor of Philosophy with a major in zoology. He worked as a graduate assistant in the Zoology Department from September, 1969, until September, 1973. He was awarded a Graduate Council Fellowship for the 1973-1974 academic year.

Charles William Leffler, II is married and the father of one child.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



F. G. Nordlie, Chairman
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



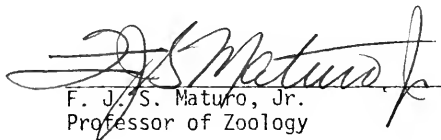
J. F. Anderson
Associate Professor of Zoology

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B. K. McNab
Professor of Zoology

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F. J. S. Maturó, Jr.
Professor of Zoology

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This dissertation was submitted to the Graduate Faculty of the Department of Zoology, in the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1974

Dean, Graduate School



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