PHYSIOLOGICAL CONSEQUENCES OF POLYCHLORINATED BIPHENYL- AND SALINITY-STRESS IN PENAEID SHRIMP

by

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INTRODUCTION

Estuaries are dynamic environments and animals that live in them must be able to cope with change. Some dynamic natural factors are temperature, salinity, currents, hydrostatic pressure, and oxygen or carbon dioxide concentrations. Unfortunately, domestic sewage (nutrients), oils, industrial chemicals, pesticides, metals, or altered temperatures are an influence in estuaries. Unfortunately, the combined effects of the natural and man-introduced factors on estuarine organisms are largely unknown. Exceptions are the estuaries which receive undue amounts of pollutants that for all practical purposes are biologically dead. In contrast, when interactions occur gradually and in combination, the effect could be the death of assemblages of organisms or an entire estuary without our being aware of the trend. Therefore, the problem facing us today is understanding and predicting the interactions of pollutants and natural stresses.

It is common knowledge that the commercial shrimps along the Gulf Coast undertake distinct euryhaline migrations. After adult shrimp spawn in the open Gulf from spring to fall, the post-mysids and juveniles migrate into the fresher waters of bays where they grow rapidly to adulthood before returning to the Gulf. Obviously, these stages of shrimp must be able to adjust to the changing salinities encountered in the estuary and this adjustment requires physiological facilities which must operate at optimum.

One group of chemicals introduced by man that has recently been the concern of many ecologists is the PCBs, or polychlorinated

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biphenyls. In 1969, a PCB, identified as Aroclor® 1254 was discovered as a contaminant in water, sediment, and fauna of Escambia Bay, Florida (Duke, Lowe and Wilson, 1970). An early survey indicated that whole body residues of the chemical in feral shrimp were as high as 14 mg/kg whole body (Nimmo et al., 1971a). Subsequent toxicity tests on juvenile pink shrimp (Penaeus duorarum) revealed that about 1.0 µg/ℓ in the water would kill 50% within 15 days (Nimmo et al., 1971b).

While conducting bioassays at our laboratory we noted on several occasions that salinity appeared to affect toxicity. In one instance, we conducted a chronic exposure in which adult pink shrimp, were exposed to a sublethal concentration of the chemical (about 1.0 µg/ℓ). The purpose of the test was to determine if structural damage might occur in gill tissues. On the 27th day of exposure at which time we had recorded no previous deaths from the PCB, the salinity of the incoming water decreased from 20 °/oo to 11 °/oo within 4 hours due to rain, tides and wind. The result was death of ten experimental shrimp before the salinity had returned to 20 °/oo . During the next two days, the salinity was lowered again by aberrant tides and climatic conditions and more experimental, but not control, shrimp died. We, therefore, became interested in the possible interaction of Aroclor® 1254 and environmental stress, particularly the effect of PCB on the ability of shrimp to regulate osmotically and ionically at reduced salinities.

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MATERIALS AND METHODS

Adult brown shrimp (Penaeus aztecus), 11.5 - 13.7 cm, rostrum to telson, were captured near Gulf Shores, Alabama and used in our studies. Approximately equal numbers of both sexes were used and the methods of exposure to the Aroclor 1254 were similar to those reported previously (Nimmo et al., 1971a). The modifications were (1) ambient salinity was maintained at 30 ± 1 °/oo and temperature, at 25 ± 2° C, and (2) the exposures to the chemical were "sublethal" and lasted but 7 days. Three µg/l was chosen as the test concentration because previous tests with adult brown shrimp as well as adult pink shrimp (P. duorarum) demonstrated that this concentration would cause 50 °/oo mortality within 30 days.

Following exposure to PCB, equal numbers of PCB-exposed and "control" shrimp were transferred to separate aquaria at ambient temperature and salinity (Fig. 1). While temperature was kept constant, the salinity in each aquarium was gradually lowered during 8 hours to a predetermined level. Since the possibility existed of physiological stress from handling or inherent in the experimental design, both PCB-exposed and control shrimp were analyzed for osmotic and ionic concentrations after going through the procedure without external salinity change (30 °/oo). For the first group, the salinity was maintained at 30 °/oo for 8 hours; the second, salinity was lowered from 30 °/oo to 22 °/oo; the third, from 30 °/oo to 10 °/oo, and the fourth, from 30 °/oo to 7 °/oo. Although there was a time differential between groups of shrimp, and therefore, a possible

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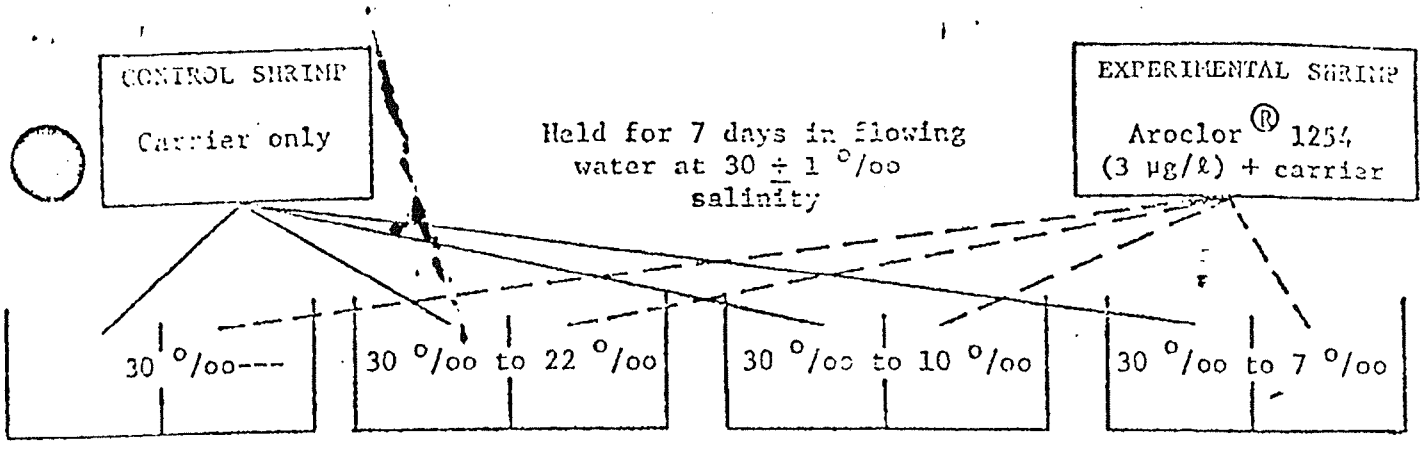

Salinity stress: flowing water with the capacity of controlled flux of 8-hr duration

Blood sample drawn from pericardial sinus, placed in glass tube, allowed to clot and osmotic concentration determined

Shrimp saved for residue analysis (GLC)

Serum separated from clot, placed in small crucible, ashed at 480 C for 6 hours and ash dissolved in 2N H_2SO_4

Determination of cations (Atomic absorption)

Determination of chloride (Coulometric titration)

FIGURE 1. FLOW SHEET FOR EXPERIMENTAL PROCEDURE

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Table 1. WHOLE BODY CONCENTRATIONS OF AROCLOR® 1254 IN PCB-EXPOSED SHRIMP*

SALINITY °/oo	mg/kg AVERAGE	RANGE
30	9.6	3.0-13.7
22	7.8	1.9-18.8
10	7.1	3.9-14.0
7	8.5	2.8-15.0

*Concentration of Aroclor® 1254 in the test water was 3 ppb; length of exposure, 7 days. Control shrimp had less than 0.1 ppm of Aroclor® 1254.

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difference in test animals due to a slight loss of PCB, analyses for the chemical revealed no significant difference in whole body concentrations among groups (Table 1). As in earlier studies, there was a wide range in individual concentrations of PCB (Nimmo et al., 1971b).

Samples of whole blood (hemolymph) for osmotic concentration were removed from each shrimp by pericardial puncture. A ground-glass syringe (1ml), fitted with a #22 gauge stainless-steel needle was inserted into the animal at an oblique angle to obtain at lease 0.3 ml of blood from each animal. The blood was immediately transferred to an osmometer tube and the osmotic concentration determined with a Fisk® Model C-66 osmometer. Since the blood clotted quickly, it was difficult to determine whether we measured osmotic concentration on whole blood or on serum, but on analyzing them separately, we found no significant difference in their osmotic concentrations. Replicate determinations of 10 separate aliquots of pooled sera from several shrimp yielded a standard error of 1.2 (mean concentration = 629 mOs).

Analyses of ions was performed on ashed sera. To prepare the sample, the clot contained in the osmometer tube was squeezed with a small glass rod, the clot was removed and exactly 0.2 ml of the serum was transferred to a small crucible. The crucible was placed in an oven and the contents ashed at 480 C for 6 hr, cooled and the ash was dissolved in 2N H_2SO_4. Analysis of chloride was performed with a Buchler-Cotlove Chloridometer® and cations were determined on a Model 403 Perkin-Elmer® atomic absorption spectrophotometer

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equipped with a deuterium arc-background corrector and an HGA-70 heated graphite atomizer. Cations in standard solutions were in the same proportions as those in the sera. As a check on our methods, an analysis of a single aliquot of serum by emission and atomic absorption yielded identical results for the element, potassium. Five replicates of pooled sera from several shrimp yielded a standard error of 0.24 mEq/l for Cl (mean = 257 mEq/l). Replicate analyses on serum aliquots yielded standard errors in mEq/l of 8.26 for Na (mean = 324.9), of 0.04 for Mg (mean = 16.0), of 0.07 for K (mean = 8.1), of 0.19 for Ca (mean = 4.74), and of 0.10 for Cu (mean = 2.84).

The 95% confidence interval was used to evaluate significance of differences in the data. The 95% intervals are indicated in the graphs by vertical bars on each datum and are listed in each table. It is sometimes difficult to relate "osmolality" or "osmotic concentration" to the environment. For the purpose of our discussion of the marine environment, we expressed the concentration of the environment as salinity. The relationship between mOs and salinity is indicated along the X axis in Figure 2.

Concentrations of Aroclor® 1254 were determined on individual shrimp by gas chromatography, using procedures summarized earlier (Nimmo et al., 1971b).

RESULTS

The most significant result of this study was the discovery that a sublethal concentration of Aroclor 1254 at constant salinity for 7 days became lethal when shrimp were subjected to a

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FIGURE 2. OSMOTIC CONCENTRATION: SERUM-ENVIRONMENT IN PENAEUS AZTECUS (after McFarland & Lee, 1963). Our study covered the range of 7 °/oo to 30 °/oo salinity.

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gradual decrease in salinity over an 8-hr period. Usually the shrimp exhibited increased swimming activity while the salinity changed from 30 °/oo to 20 °/oo, and this was the only behavioral aberration observed. Deaths in experimental shrimp began at a salinity of about 12-13 °/oo and at 10 °/00 and 7 °/oo, when 8 hours had elapsed, mortality of experimental shrimp was nearly 50%. When 50% of the experimental shrimp had become moribund or had died, living PCB-exposed shrimp were taken for analyses of osmotic concentrations and ion determinations on sera.

The results of these analyses indicated that concentrations of most major ions in the sera of PCB-exposed shrimp became significantly less as the ambient salinity decreased, the sum of major ions (e.g., Na, Ca, Mg, K, Cu, and Cl) being 18% less after ambient salinity reached 10 °/oo or 7 °/oo (Fig. 3). Of this total, sodium was 16% less (Fig. 4), chloride, 19% (Fig. 5) and calcium, 25% (Fig. 6). There was some indication that magnesium decreased, but not significantly (Table 2). No apparent differences in potassium (Table 3), or copper (Table 4) were noted.

Data for iron (Table 5) are not included in the totals in Fig. 3 because we could not distinguish the divalent from the trivalent form.

Despite significant alterations in the major ion complement or in some major ions, osmotic contentration was not significantly effected by PCB and salinity stress (Table 6). Seemingly, osmotic pressure was less in PCB-exposed shrimp at 10 °/oo or 7 °/oo

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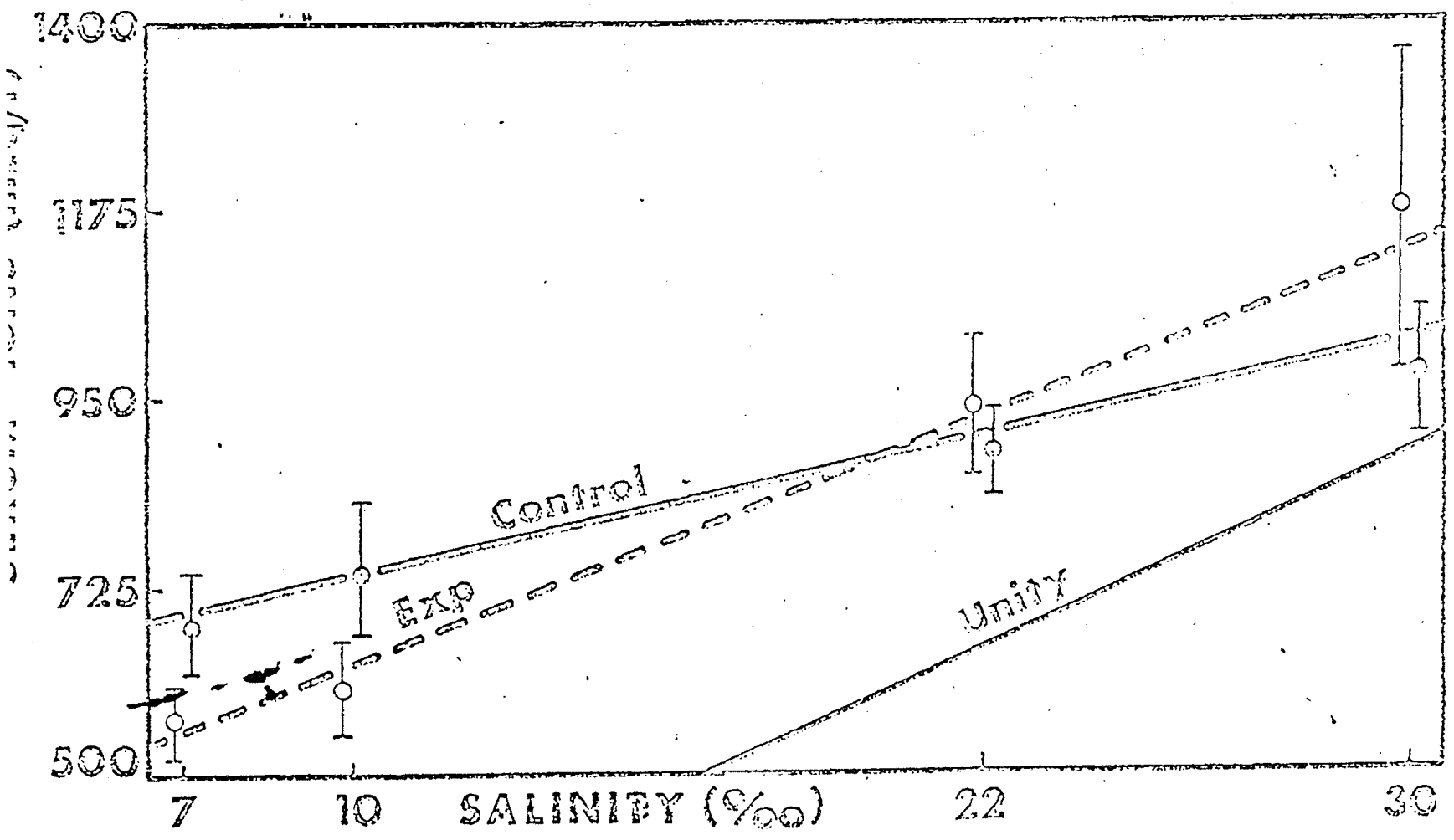

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FIGURE 3. TOTAL IONS IN BROWN SHRIMP SERUM IN RELATION TO SALINITY

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FIGURE 4. SERUM SODIUM IN BROWN SHRIMP IN RELATION TO SALINITY

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FIGURE 5. SERUM CHLORIDE IN BROWN SHRIMP IN RELATION TO SALINITY

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FIGURE 6. SERUM CALCIUM IN BROWN SHRIMP IN RELATION TO SALINITY

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Table 2. AVERAGE SERUM CONCENTRATIONS OF MAGNESIUM IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY	mEq/l			
°/oo	CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
30	20.1	16.7--23.5	17.3	12.7--21.8
22	16.3	14.2--18.4	14.6	12.3--16.9
10	14.8	12.4--17.2	13.0	11.0--15.0
7	15.0	11.7--18.3	11.5	8.9--14.2

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Table 3. AVERAGE SERUM CONCENTRATIONS OF POTASSIUM IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY °/oo	mEq/l CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
30	14.2	13.5--14.9	14.7	13.6--15.7
22	11.7	11.1--12.3	12.1	11.2--12.9
10	10.4	9.8--11.2	10.1	8.7--11.4
7	9.6	8.4--10.7	10.0	8.4--11.6

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Table 4. AVERAGE SERUM CONCENTRATIONS OF COPPER IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY	mEq/l			
	CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
°/oo				
30	4.7	3.6--5.9	5.3	4.4--6.3
22	4.0	3.4--4.6	5.9	4.8--7.0
10	5.2	4.0--6.4	4.6	3.8--5.3
7	4.8	3.9--5.7	5.7	5.0--6.4

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Table 5. AVERAGE SERUM CONCENTRATIONS OF IRON IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY °/oo	mM/l CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
30	.23	.17--.29	.31	.15--.48
22	.28	.15--.35	.18	.14--.22
10	.20	.15--.25	.26	.17--.35
7	.24	.14--.33	.33	.24--.41

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Table 6. AVERAGE SERUM OSMOTIC CONCENTRATIONS IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY	MILLIOSMOLES			
	CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
°/oo				
30	749	728--771	752	726--779
22	687	665--708	688	658--718
10	551	508--593	523	500--546
7	547	521--572	516	495--537

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salinity but individual variation was too great to show a significant difference from controls.

DISCUSSION

Knowledge of interactions between toxic compounds and environmental factors is essential for predicting their effects on ecosystems or species. Examples of this need have been demonstrated in both fresh and marine investigations. In fresh water, low-level chronic exposure of the darter (Etheostoma nigrum) to dieldrin greatly affected its ability to survive thermal stress (Silbergeld, 1973). The sublethal effects of mercury on fiddler crabs (Uca pugilator) reduced survival times when crabs were placed under temperature and salinity stress (Vernberg and Vernberg, 1972). Mortality of fiddler crabs previously exposed to cadmium was greatest at high temperatures and low salinites (O'Hara, 1973).

In our studies Aroclor® 1254 possibly interfered with the adenosine triphosphatase (ATPase) activity in gills of shrimp. ATPase activity is associated with active ion transport (Tanaka, Sakamoto and Sakamoto, 1971). Polychlorinated insecticides and the related polychlorinated biphenyls have been shown by several "in vitro" assays to inhibit ATPase in the tissues of fishes (Davis and Wedemeyer, 1971a; 1971b; Davis, Friedhoff and Wedemeyer, 1972: Cutkomp et al., 1972; Yap et al., 1971) and in the nerves of lobsters (Matsumura and Narahashi, 1971). The need for greater efficiency or capacity of ATPase in marine organisms can be inferred from the results of

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Pfeiler and Kirschner (1972), who showed that gill ATPase activity of rainbow trout adapted to salt water was greater than in fish adapted to fresh water.

Polychlorinated hydrocarbons have interferred with either osmo- or ionic-regulation in aquatic animals (Eisler and Edmunds, 1966; Grant and Mehrle, 1970; Kinter et al., 1972; Nimmo and Blackman, 1972). The physiological relationship of ionic effects to that of ATPase activity was first reported by Kinter et al. (1972), who postulated that lipophilic agents such as DDT and PCBs, might interact with the phospholipid-activating components of the lipoprotein enzyme. The effect of dieldrin on ion movement in the nervous system of cockroaches showed that dieldrin inhibited binding of calcium to the phospholipid moiety of the enzyme, thus inhibiting the movement of calcium across the nerve membrane (Hayashi and Matsumura, 1967). Calcium salts in fresh water greatly increased the ability of marine and euryhaline animals to survive in that medium (Black, 1957). Toxic symptoms of DDT poisoning in fresh water fish could be alleviated by the addition of calcium salts (Keffler, 1972). In our studies calcium was lowered 25% in the sera of PCB-exposed shrimp at 7 °/oo salinity, and it may be that this reduction was responsible in part for the observed decrease in sodium, chloride, and other ions.

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Field observations of juvenile and subadult brown shrimp by several investigators indicate that those shrimp tolerate a wide range of salinities:

Salinity	Location	Observer
0.22 - 0.36 °/oo	St. Lucie estuary, Fla.	Gunter and Hall (1963)
0 - 1.0 °/oo	Mobile Bay, Ala.	Loesch (In Gunter et al. 1964)
0 - 2.0 °/oo	Choctawhatchee Bay, Fla.	Nimmo (unpubl. data)
69 °/oo	Laguna Madre, Texas	(1957)

Gunter, Christmas, and Killebrew (1964), found that in Texas bays, young brown shrimp were most abundant at 10 to 30 °/oo, with greater abundance above 20 °/oo. Zein-Eldin and Aldrich (1965) found that postlarval brown shrimp withstood a wide range of salinity-temperature combinations.

It is evident that adult shrimp are osmoregulaters at all but extremes of salinity (Fig. 2). Williams (1960) gives the isosmotic point of shrimp hemolymph at 26.5 °/oo (788 mOs), as compared to our calculation of 23.4 °/00 (694 mOs) in control shrimp. Nevertheless, there was no significant difference in PCB-exposed and control shrimp (Table 6). Obviously, a slight change in environmental osmotic pressure would not be critical to the osmoregulatory ability of the animals at isosmoticity, but was critical in the dilute environment. Therefore, the salinities where the PCB exerted its

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greatest effect in the laboratory (as judged from mortality of shrimp) were well within the range in which brown shrimp occur in nature.

Although no appreciable difference occurred in osmotic concentration, there was significant difference in major ions in the sera of PCB-exposed shrimp (Fig. 3). McFarland and Lee (1963) found that the point of convergence of the total ions in the hemolymph of feral shrimp to that of the environment was 27 °/oo (800 mOs). In our study, by extrapolation, convergence occurred at 34 °/oo (1000 mOs) in controls, whereas in PCB-exposed shrimp showed no convergence and total ions paralleled unity.

In surveys conducted soon after PCB was first discovered in the Pensacola estuary, the distribution of shrimp in relation to salinity seemed to be related the amount of the chemical in the animals (Nimmo et al., 1971b). Of three species captured, brown shrimp had the highest whole-body residues (14 ppm) although most samples were lower. Seemingly, a concentration of 14 ppm PCB in feral shrimp would have been lethal if the animals were subjected to salinity stress such as imposed by our experimental procedure. We have unpublished data that suggest existence of a threshold in average whole-body concentration of PCB (5.6 to 7.8 mg/kg) in pink shrimp (P. duorarum) that would be lethal when superimposed on salinity stress caused by our procedure. However, a recent survey of feral shrimp from the Pensacola estuary showed that young adult shrimp now have only a fraction of PCB concentrations found in 1969/70

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periods. For example, a sample taken from Escambia Bay in August, 1973 had a whole-body concentration of only 0.1 mg/kg.

Future studies should include research on interaction of PCB and salinity on juvenile and postlarval shrimp since chronic toxicity tests have shown that these stages were more susceptible to the chemical (Nimmo et al., 1971a). In addition, studies by Dana Beth Tyler-Schroeder, of the Gulf Breeze Laboratory, have shown the susceptibility of larvae of grass shrimp (Palaemonetas pugio) to the PCBs, Aroclors® 1016 and 1242, decreases with age (personal communication). Also, the "in vivo" effect of PCBs on ATPase activity in shrimp should be fully investigated.

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