Marine Biology 92, 245–254 (1986)

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Primary and secondary stress responses of striped bass (*Morone saxatilis*) exposed to benzene

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Abstract

Striped bass (*Morone saxatilis*) were collected from the San Francisco Bay-Delta estuary in 1982, from areas distant from pollution sources. After acclimatization, plasma cortisol concentration (primary response) and blood biochemicals indicative of energy mobilization (secondary responses) were followed in individuals exposed to sublethal levels of benzene for up to 21 d. Despite the persistence of benzene in blood and liver tissues for the exposure duration, stress responses were moderate and returned to control values within the initial 7 d. Blood and liver rapidly accumulated benzene to approximately 20 times the exposure concentrations (0.1 and 1.0 ppm). Concentrations of cortisol and secondary response variables were not proportional to benzene exposure or to accumulation levels, however. Plasma cortisol concentrations increased two- to three-fold at 8 h and returned to control levels prior to 48 h exposure. Glucose, lactate, H^+, protein and triglyceride concentrations were elevated during the initial 4 h to 7 d, with protein and triglyceride returning to normal levels prior to the other secondary response variables. From the perspective of the general adaptation syndrome (GAS), benzene activated the hypothalamo-pituitary-interrenal axis, resulting in clinical stress responses characteristic of an alarm reaction. The time-courses and amplitudes of primary and secondary responses to benzene suggest the sensory perception of a noxious agent eliciting mild, acute stress followed by adaptation.

Introduction

The striped bass (*Morone saxatilis*) population of the San Francisco Bay-Sacramento River and San Joaquin River Delta estuary has declined by approximately 75% since the early 1960's (Stevens *et al.*, 1985). This decline has been attributed to any or all of four factors: reduced egg production, inadequate food supply for young striped bass, entrainment losses by water diversions, and pollution (Kelley, 1982). Studies have found high concentrations of organochlorines, metals and petrochemicals in striped bass tissues (Gadbois and Maney, 1983; Whipple *et al.*, 1983). Two-thirds of the striped bass sampled in 1978 contained benzene, a little-studied toxic aquatic contaminant, in liver tissue to approximately 4 ppm (Whipple *et al.*, 1983). These fish were in relatively poor physiological and reproductive condition, were heavily parasitized and exhibited a unique immune response to parasite infestations. In the most severe cases, this response was characterized by open external lesions which exposed internal organs (Moser *et al.*, 1985).

It is well established that fish exhibit characteristics of the general adaptation syndrome (GAS) (Selye, 1950) in response to a variety of stressors including many pollutants (Donaldson, 1981). The initial or alarm phase of the GAS is characterized by elevated plasma levels of corticosteroids and catecholamines which mediate secondary metabolic and physiological responses. These responses include mobilization and catabolism of lipids, proteins, and carbohydrates (Mazeaud *et al.*, 1977). Metabolic alterations have evolved as adaptive mechanisms to provide physiological compensation during suboptimal conditions. If adaptation occurs during continuous or repeated stressful conditions, homeostasis is regained and circulating levels of the "stress" hormones return to normal. However, if adaptation does not occur, the organism progresses into the exhaustion stage of the GAS, producing deleterious consequences such as reduced growth, reproductive impairment, immunological dysfunctions and, ultimately, death.

The purpose of the present investigation was to determine whether benzene exposure produces alterations of plasma cortisol, a primary stress response, and blood biochemicals indicative of energy mobilization in striped bass. Energy mobilization and metabolic effects of ben-

zene exposure were evaluated by measuring circulating levels of glucose, lactate, H^+, triglycerides, and protein which have been shown to be responsive to stressors (Mazeaud and Mazeaud, 1981; Wedemeyer and McLeay, 1981).

Materials and methods

Morone saxatilis (2.9 ± 0.6 yr old; 36.9 ± 5.4 cm standard length; 944 ± 342 g wet weight) were obtained by gill net and trawl from areas of the San Francisco Bay-Delta estuary distant from point sources of pollutants and were held in the laboratory for one to six months to acclimatize and eliminate any contaminants accumulated in the environment susceptible to depuration. The physiological condition of the experimental fish was assessed according to the procedures of Whipple *et al.* (1984) and was found to be representative of the healthier end of the spectrum in the Bay-Delta population, providing suitable subjects upon which to evaluate the effects of benzene exposure.

Striped bass were held in a 20 000-liter fiberglass raceway receiving filtered bay water (approximately 25‰ S and 15 °C) under ambient light conditions until 2 wk prior to experimentation (September, 1982), when they were transferred to the experimental tanks. Ten fish were randomly assigned to each of nine 2,200-liter circular fiberglass aquaria and acclimatized to filtered bay water flowing at a rate of 7 liters min^{-1} (27.5 ± 1.7‰ S, 17.1 ± 1.3 C°). Photoperiod in the experimental tanks was adjusted weekly to the natural cycle. Each tank was partially covered to minimize disturbance. Water quality measurements and behavioral observations were made daily throughout the acclimatization and experimentation periods. Dissolved oxygen was monitored with a calibrated YSI Model 54 oxygen meter (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA) and had a mean value of 7.3 ± 0.5 ppm. Unionized ammonia was measured at < 0.002 ppm by an Orion Model 95-10 ammonia electrode (Orion Research Inc., Cambridge, Massachusetts) corrected for temperature and pH. Fish were fed chopped squid twice weekly until satiated during acclimatization and experimentation, but never within 72 h of sampling to limit any possible contribution of feeding schedule to cortisol level (Delahunty *et al.*, 1978). There were no mortalities during either period.

Three of the experimental aquaria received water with a benzene concentration of 0.1 ppm (volumetric) calculated to produce liver benzene levels similar to those of striped bass collected from the San Francisco Bay-Delta. An additional three aquaria received water with a 1.0 ppm benzene concentration to amplify biological responses. Finally, three control aquaria received no benzene. Although rarely measured in the aquatic environment because of sampling and analytical difficulties, benzene is the most abundant aromatic hydrocarbon in the water-soluble fraction of certain crude oils (Anderson *et al.*, 1974), a major component of gasoline (Cornish, 1980), and has a solubility of 1 400 $\mu l\ l^{-1}$ in 25‰ S seawater at 16 °C (Benville and Korn, 1977). Based on data from the literature (Korn *et al.*, 1977; Whipple *et al.*, 1981), we believe 0.1 ppm to be a high environmental concentration. Within the proximity of a gasoline or petroleum spill, levels could equal or exceed 1.0 ppm (Hirsch *et al.*, in press). Both exposure concentrations were well below the 96 h LC_{50} (the concentration lethal to 50% of the test subjects within a specified time period) of 10.9 ppm for striped bass in a continuous-flow system (Meyerhoff, 1975). Initial benzene concentrations were rapidly produced by adding an appropriate volume of stirred, saturated benzene in distilled water stock solution (1 900 $\mu l\ l^{-1}$) to each aquarium. Concentrations were maintained by metering the benzene solution through Teflon tubing into the line delivering bay water to each tank. Benzene concentrations were determined twice daily using a Hewlett-Packard 5880 gas chromatograph (Hewlett-Packard, Avondale, Pennsylvania, USA) (Benville *et al.*, 1981). Charcoal filters were fitted to aquaria effluent lines to remove benzene.

Striped bass were exposed to benzene for 4 to 504 h (21 d). At each sampling time, one fish was removed from each tank to reduce capture-induced stress responses in other fish due to disturbances (as evidenced in brown trout by Pickering *et al.*, 1982). Within 2 min of initial disturbance, blood was withdrawn from each fish by cardiac puncture into Vacutainers (Becton Dickinson, Rutherford, New Jersey, USA) with and without heparin. One ml whole blood was immediately pipetted into 0.5 ml TF Freon, capped, and shaken vigorously for subsequent benzene analysis. Blood benzene concentrations were measured by injecting 3.2 μl of the Freon layer into the gas chromatograph using nonane as an internal standard. Samples for whole-blood lactate determination were prepared by pipetting 0.3 ml blood into 0.6 ml of 8% perchloric acid (weight/volume) at 4 °C and analyzed within 24 h according to the instructions supplied with the Sigma lactic acid test kit (Sigma Chemical Co., St. Louis, Missouri, USA). The pH of the blood was measured, followed by plasma isolation by centrifugation at $1\,600 \times g$ for 10 min. Portions of plasma were then stored at −85 °C for subsequent glucose and triglyceride analyses. The remaining plasma was extracted for corticosteroid determination using C_{18} Sep-Pak cartridges according to the procedure of MacFarlane (1984), and stored at −20 °C. Serum separated from unheparinized blood samples was stored at −20 °C for protein analysis.

After blood collection, liver was excised and analyzed for benzene as described by Whipple *et al.* (1978). Corticosteroids were eluted from the Sep-Pak cartridges and determined by high-performance liquid chromatography (MacFarlane, 1984). Plasma glucose was measured by the hexokinase/glucose-6-phosphate dehydrogenase method using the Sigma test kit. Plasma triglyceride determination was accomplished by the enzymatic method of Bucolo and David (1973), which measures glycerol liberated by lipase hydrolysis, ultimately coupled to the oxidation of NADH.

The Lowry method was used for total serum protein determination (Lowry *et al.*, 1951). Statistical analysis of the data to evaluate the effects of benzene concentration and exposure time was performed by a fixed-effect, two-way analysis of variance (ANOVA) and covariance and by Duncan's multiple range test (Duncan, 1955) at significance levels of 0.10, 0.05 and 0.01 using SPSS statistical analysis computer programs (Nie *et al.*, 1975).

Results

Considering the volatility of benzene, the dosing system provided stable concentrations in the aquaria (Fig. 1). All tanks attained benzene concentrations within 10% of the targeted values within 1 h of benzene addition. The mean benzene concentrations for the 21 d experimental duration were 0.113 ± 0.006 ppm and 1.008 ± 0.148 ppm for the low and high exposures, respectively. The only difficulty occurred between 7 and 14 d exposure when, in two of the high-exposure tanks, a benzene-consuming microbial (presumably bacterial) growth developed and reduced the concentration. This was corrected by spiking the tanks with the appropriate volume of a saturated benzene solution via a siphon placed beside the inflowing water until the desired concentration was regained. During this period, dissolved oxygen declined to 5.0 ppm but no signs of respiratory distress in *Morone saxatilis* were observed.

Benzene concentrations in blood revealed rapid uptake, attaining maximal levels by 8 h in the low exposure and 24 h in the high exposure (Fig. 1). The variability of benzene concentrations in blood among fish at each exposure level and sampling time was low. However, the decline of benzene in tanks with microbial blooms resulted in significant decreases in blood benzene concentrations in fish at 7 and 14 d. Regression of blood benzene level at the time of sacrifice on the water benzene level for all exposed fish revealed that blood concentrates benzene to a steady state of approximately 20 times the water concentration ($[\text{benzene}_{\text{blood}}] = 0.13 + 19.93\ [\text{benzene}_{\text{water}}]$, $r = 0.96$, $n = 42$).

Liver accumulated high concentrations of benzene (Fig. 2). Exposure to 0.1 ppm benzene achieved tissue levels within the range observed in livers of field specimens of Bay-Delta striped bass. Similar to blood, uptake was rapid and maximum concentrations were attained sooner in the low-exposure than in the high-exposure tanks. The decline of liver benzene in the 1.0 ppm exposure followed the declines of benzene in the water and blood. Regression analysis revealed that benzene concentrations in liver were essentially the same as in blood for all exposure durations ($[\text{benzene}_{\text{liver}}] = 0.23 + 0.93\ [\text{benzene}_{\text{blood}}]$, $r = 0.90$, $n = 42$). This relationship indicates that the liver does not accumulate unmetabolized benzene above the concentration found in blood. Within the exposure duration to the benzene concentrations tested,

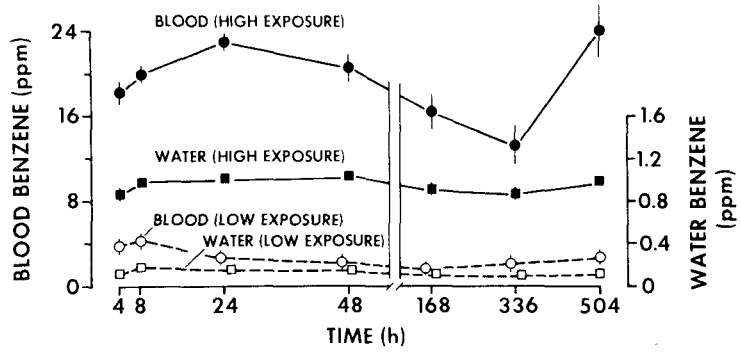

Fig. 1. *Morone saxatilis.* Benzene concentrations in exposure tanks and blood. Targeted benzene concentrations in water were 0.1 and 1.0 ppm for low and high exposures, respectively. Values represent means ± SE; where error bars are not shown, SE was less than size of symbol

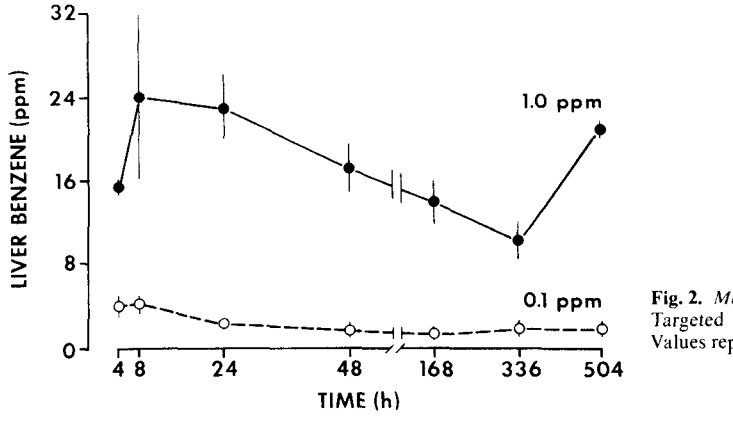

Fig. 2. *Morone saxatilis.* Accumulation of benzene in liver. Targeted benzene-exposure concentrations are indicated. Values represent means ± SE

Table 1. *Morone saxatilis.* Results of a fixed-effect, two-way analysis of variance for changes in blood variables at three levels of benzene exposure (0, 0.1 and 1.0 ppm) for seven exposure durations up to 504 h; DF: degrees of freedom; MS: mean square

Variable	Source	DF	MS	*F* ratio
Cortisol	Main effects	8	4 203.00	8.92**
	[benzene]	2	1 972.36	4.18*
	time	6	4 947.70	10.50**
	Two-way interaction	12	3 298.13	7.00**
	Residual	41	471.37	
	Total	62	1 570.07	
Glucose	Main effects	8	2.19	11.31**
	[benzene]	2	5.03	25.97**
	Time	6	1.25	6.45**
	Two-way interaction	12	0.42	2.14*
	Residual	41	0.19	
	Total	62	0.52	
Lactate	Main effects	8	0.94	16.21**
	[benzene]	2	2.19	37.61**
	time	6	0.52	8.95**
	Two-way interaction	12	0.11	1.89
	Residual	41	0.06	
	Total	62	0.18	
pH	Main effects	8	0.05	17.79**
	[benzene]	2	0.09	35.26**
	time	6	0.03	11.75**
	Two-way interaction	12	0.003	1.16
	Residual	41	0.003	
	Total	62	0.008	
Protein	Main effects	8	0.89	6.03**
	[benzene]	2	2.19	14.82**
	time	6	0.46	3.08*
	Two-way interaction	12	0.29	1.97
	Residual	41	0.15	
	Total	62	0.32	
Triglyceride	Main effects	8	44 034.35	7.71**
	[benzene]	2	27 075.27	4.74*
	time	6	49 164.79	8.60**
	Two-way interaction	12	10 321.42	1.81
	Residual	41	5 714.97	
	Total	62	11 726.79	

* $P<0.05$; * $P<0.001$

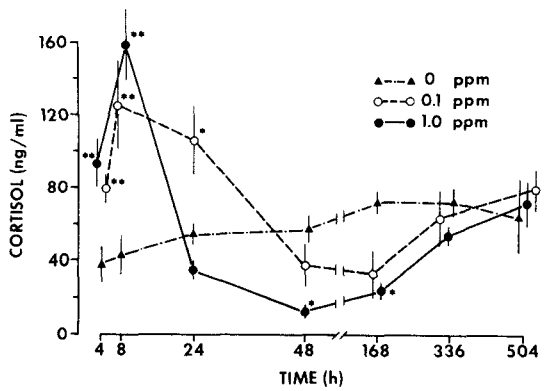

Fig. 3. *Morone saxatilis.* Plasma cortisol concentrations in benzene-exposed and control striped bass. Values represent means ±SE. Asterisks denote values significantly different from same-time control values (Duncan's multiple range test: * $P<0.05$; ** $P<0.01$)

the concentration of benzene in the blood was a linear function of benzene level in the water and benzene in the liver was a linear function of blood benzene-level.

Cortisol was the only corticosteroid detected in the plasma of the striped bass. Both benzene concentrations produced elevated cortisol levels within 4 h of exposure and maximal responses at 8 h (Fig. 3). Cortisol responses to both benzene levels at 4 and 8 h were similar and were not proportional to exposure concentrations. Cortisol declined after 8 h, with a greater decline in the 1.0 ppm exposure. Control cortisol levels were attained by 24 h in 1.0 ppm benzene, but were not achieved until 48 h in the 0.1 ppm concentration. Analysis of variance revealed a significant interactive effect of benzene on plasma cortisol ($P<0.001$) (Table 1). Comparison of benzene-exposed fish with same-time controls by Duncan's multiple range test revealed that cortisol concentrations were significantly greater in fish exposed to both benzene levels at 4 and 8 h

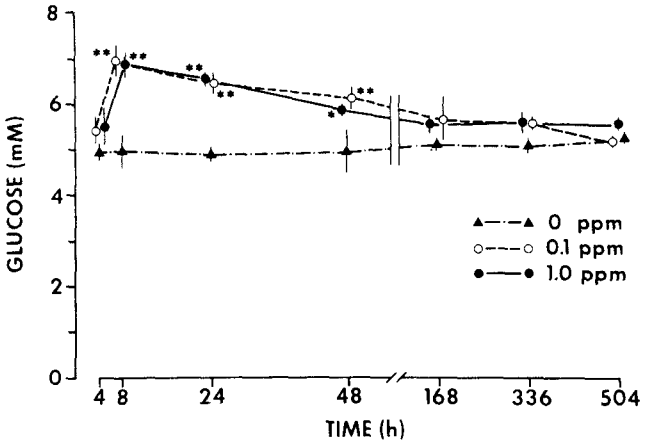

Fig. 4. *Morone saxatilis.* Plasma glucose concentrations in benzene-exposed and control striped bass. Further details as in Fig. 3

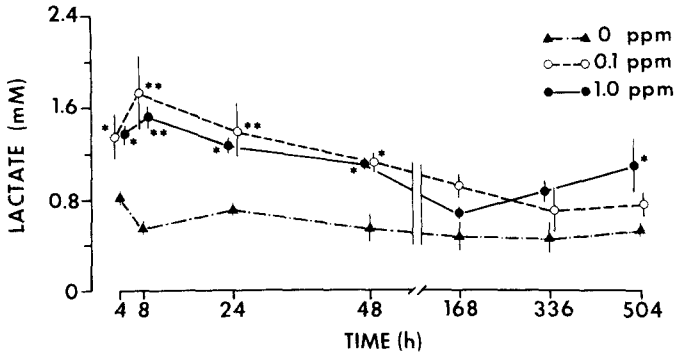

Fig. 5. *Morone saxatilis.* Blood lactate concentrations in benzene-exposed and control striped bass. Further details as in Fig. 3

($P < 0.01$) and at 24 h in the 0.1 ppm benzene exposure ($P < 0.05$). Cortisol concentrations were significantly lower, however, at 48 h and 7 d in 1.0 ppm benzene ($P < 0.05$) compared to same-time controls. At the 0.05 significance level, mean cortisol values of controls at all sampling times were not significantly different.

Elevated plasma glucose developed later and persisted longer than the cortisol response in both benzene concentrations (Fig. 4). Hyperglycemia was not evident until 8 h exposure and control values were not re-established until after 48 h. Responses were essentially identical for both exposure concentrations, with maximal concentrations recorded at the same sampling time as for cortisol. Analysis of variance showed a significant interactive effect of benzene level and exposure time on plasma glucose ($P < 0.05$) (Table 1). Exposure to both benzene concentrations resulted in significant glucose elevations compared to control values at 8, 24, and 48 h.

Blood lactate concentrations were significantly elevated for the first 48 h in fish exposed to both benzene levels (Fig. 5). The time-course of the response was similar to that of glucose and was not dose-dependent. However, unlike glucose, lactate was significantly greater at 4 h exposure compared to controls, and there was a secondary elevation at 21 d exposure to 1.0 ppm benzene. Both main effects were statistically significant ($P < 0.001$) (Table 1).

Striped bass exposed to benzene exhibited lower blood pH than controls (Fig. 6). The greatest depressions of blood pH occurred during the first 8 h in both treatments and returned to control values by 7 d exposure. However, there was a secondary pH decrease in the 1.0 ppm benzene exposure by 14 d that corresponded to increasing lactate. ANOVA revealed significant individual main effects of benzene concentration and exposure time ($P < 0.001$) similar to lactate (Table 1). The association between blood lactate and pH observed in these experiments indicated metabolic acidosis and is supported by the correlation of H^+ and lactate concentration, $r = 0.88$, $n = 42$, $P < 0.001$.

Benzene exposure produced a moderate elevation in serum protein (Fig. 7). Protein was maximal at 4 h exposure to 1.0 ppm benzene and at 8 h in 0.1 ppm media. Control levels were established by 24 to 48 h with no further responses evident for the duration of the exposures. ANOVA revealed significant individual main effects of benzene concentration and exposure duration (Table 1).

Plasma triglyceride responded rapidly to benzene exposure, attaining approximately 550 mg dl^{-1} at 4 h, but

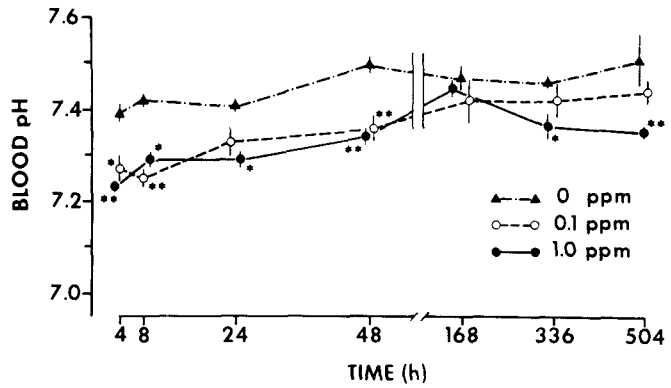

Fig. 6. *Morone saxatilis.* Blood pH in benzene-exposed and control striped bass. Further details as in Fig. 3

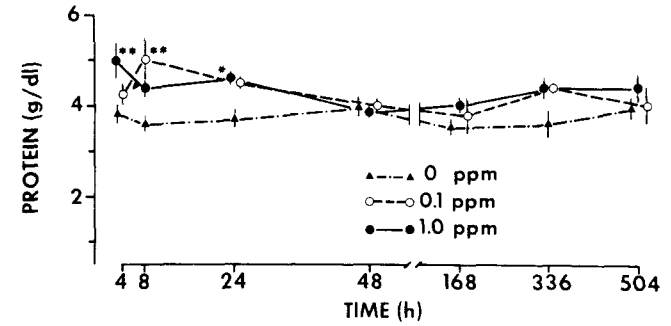

Fig. 7. *Morone saxatilis.* Serum protein concentrations in benzene-exposed and control striped bass. Further details as in Fig. 3

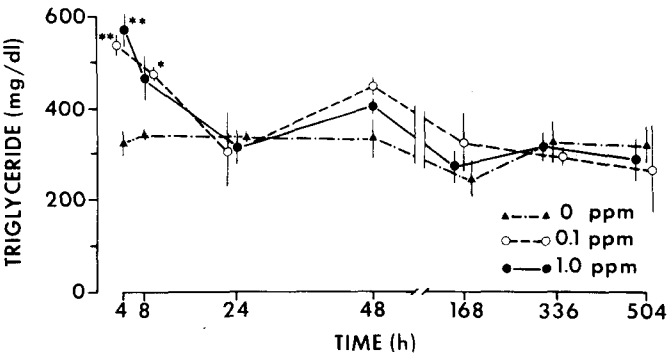

Fig. 8. *Morone saxatilis.* Plasma triglyceride concentrations in benzene-exposed and control striped bass. Further details as in Fig. 3

rapidly declined to control levels within 24 h (Fig. 8). Similar to the other biochemical responses, plasma triglyceride was not dose-dependent; both benzene concentrations evoked similar elevations and time-courses.

Alterations in behavior were observed in striped bass exposed to benzene. During the initial 4 d, exposed fish swam more continuously, appeared much more restless and displayed greater frequency and amplitude of opercular movements than controls. These responses were greater in the 1.0 ppm benzene solution than in the lower concentration. From Day 4 through Day 7, exposed striped bass performance was more similar to that of controls. After 7 d, fish in benzene-dosed water became increasingly hyperactive, particularly in the high benzene exposure.

Feeding was on a random schedule to eliminate food-induced effects on blood variables and was never within 3 d prior to sampling. Fish exposed to both benzene concentrations ate well when food was presented on Day 2 after sampling. After 7 d exposure, those in 1.0 ppm benzene ate approximately 50% of the ration within 2 h of presentation. By 14 d exposure, only about 10% of the food was consumed. Striped bass in 0.1 ppm benzene consumed about 75% of the ration on Day 7 within 1 h of presentation; thereafter, they ate less than 50% of the ration over a several-hour period. In contrast, controls always consumed 100% of the ration within 10 to 30 min.

Discussion

The time-courses of the cortisol and secondary metabolic responses of *Morone saxatilis* to benzene exposure are characteristic of mild, acute stress elicited by the sensory

perception of a noxious agent. These responses, typical of the alarm phase of the general adaptation syndrome (Selye, 1950), were followed by a return to control levels within 2 to 7 d despite the persistence of benzene in blood and liver for the 21 d duration of exposure. Thus, the resistance phase of the GAS had occurred. These results are congruous with the concept discussed by Schreck (1981) that the initial moments during encounter with a stressor that causes fright, pain or discomfort are the most important in establishing clinical stress responses. This phenomenon has been observed with other fish challenged by chemical stressors (Schreck and Lorz, 1978; Thomas and Neff, 1985). Sensory perception of benzene is supported by the recent study of Babcock (in press). When exposed to sublethal concentrations of benzene, pink salmon (*Oncorhynchus gorbuscha*) olfactory rosettes contained exhausted mucous cells, indicating olfactory stimulation.

Uptake and equilibrium of benzene in the blood were rapid. The major uptake pathway for benzene appears to be across the gill membrane with subsequent sorption by blood cells and perhaps lipoproteins (Gerarde, 1960; Whipple *et al.*, 1981). Benzene removal from blood was in a steady state with liver uptake, as revealed by the relationship of benzene concentrations in blood and liver. Liver is a major site of benzene accumulation and metabolism in striped bass (Korn *et al.*, 1976; Whipple *et al.*, 1981) and other teleosts (Korn *et al.*, 1977; Roubal *et al.*, 1977). Liver benzene concentrations in fish exposed to 0.1 ppm were within the concentration range found in livers of striped bass obtained from the San Francisco Bay-Delta (0.02 to 4.02 ppm, $N=105$, Whipple *et al.*, 1983), justifying this exposure concentration as a valid environmental level.

Rapid metabolism of benzene was suggested in the present study by the steady-state relationship between benzene concentrations in blood and liver and by the coincidental declines of benzene in water and liver during the microbial blooms. Radiolabelled tracer studies suggest that benzene is metabolized in hepatic tissue, stored in the gall bladder, and excreted in the feces and urine (Korn *et al.*, 1976, 1977; Roubal *et al.*, 1977). As in mammals, hepatic detoxification is probably accomplished by hydroxylation reactions by cytochrome P-450 and the mixed function oxygenase (MFO) system to form phenol, catechol and hydroquinone, followed by the formation of glucuronide and sulphate conjugates (Jerina and Daly, 1974; Snyder and Kocsis, 1975).

Activation of the hypothalamo-pituitary-interrenal axis had occurred prior to the first sampling at 4 h exposure to benzene. Plasma cortisol was elevated during the initial period of exposure but returned to control, or lower, values despite the continued presence of the stressor, as observed in other studies (Donaldson and Dye, 1975; Schreck and Lorz, 1978; Leach and Taylor, 1980; Thomas *et al.*, 1980, 1981; Tomasso *et al.*, 1981). From the perspective of the GAS, responses of this type suggest that the animal is in the process of adapting to an altered environment following a period of alarm. This is in contrast to the results of other studies where cortisol remained elevated throughout the duration of stressor exposure (DiMichele and Taylor, 1978; Barton *et al.*, 1980). The former pattern appears more common, suggesting that fish can generally adapt to mild, chronic stressors. Of course, this ability is influenced by many factors such as the type, duration and intensity of the stressor; the life stage; and the presence or absence of other stressing agents. Thomas *et al.* (1980) conjectured that volatile compounds including benzene, in addition to naphthalene, were involved in the acute elevation of plasma cortisol in *Mugil cephalus* exposed to a 20% water-soluble fraction of No. 2 fuel oil. The present study supports that conjecture. Due to the method of exposure, Thomas *et al.* (1980) were unable to establish whether adaptation of the cortisol response to aromatic hydrocarbons occurred. Cortisol titers in *Fundulus heteroclitus* after 15 d exposure to naphthalene suggested that adaptation did not occur (DiMichele and Taylor, 1978). Our study indicates adaptation does occur, at least in striped bass. Of course, longer exposure resulting in loss of adaptation and therefore exhaustion can not be excluded.

In striped bass exposed to 1.0 ppm benzene, the decline of cortisol to levels below those of controls could be attributed to several processes including interrenal exhaustion, feedback inhibition of ACTH production, or enhanced metabolism of cortisol. The first two possibilities are unlikely since the duration of the cortisol response was shorter than has been recorded from other studies (DiMichele and Taylor, 1978; Schreck and Lorz, 1978) and the amplitude of the cortisol response was much less than the response produced by confinement stress (MacFarlane, 1984). Enhanced hepatic metabolism of cortisol in benzene-exposed fish was more likely the cause of the decline. Although not evaluated in the present study, benzene induction of the mixed function oxygenase (MFO) system, known to occur in mammals (Snyder and Kocsis, 1975), may account for reduced plasma levels of cortisol. There is evidence that the same MFO system can metabolize both steroids and xenobiotics in birds and mammals (Kupfer and Peets, 1966; Conney, 1967; Nowicki and Norman, 1972) and that such stimulation of cortisol metabolism can result in depletion (Kupfer, 1975). Sivarajah *et al.* (1978) found a correlation between increased hepatic microsomal enzyme activity and decreased plasma steroids in *Cyprinus carpio* and *Salmo gairdneri* exposed to the PCB, Aroclor 1254. Induction of this detoxification mechanism is rapid. Lee *et al.* (1972) demonstrated that metabolism of naphthalene was essentially complete within 24 h of exposure in three species of marine fishes.

Elevated plasma corticosteroids and catecholamines promote an array of secondary metabolic responses to adapt an animal to an altered environment (Selye, 1950; Donaldson, 1981). In the present study, the time-courses of blood variables indicative of energy mobilization and utilization were consistent with the cortisol response and indicated initial, transitory stress followed by adaptation to benzene exposure within 7 d. During the alarm phase, responses to both benzene concentrations were essentially

similar. Furthermore, hematological measurements (e.g. hematocrit, hemoglobin, etc.) revealed that plasma levels of blood biochemicals were not attributable to hemoconcentration. The effects of benzene on striped bass hematology will be the subject of a future paper (MacFarlane, unpublished data).

The rise in plasma glucose, often observed in teleosts in response to stressors (Silbergeld, 1974; Mazeaud *et al.*, 1977), succeeded the cortisol response and persisted for longer than 48 h. Since glucose mobilization is promoted by cortisol and catecholamines, this pattern is not unexpected and was similar, but of lower magnitude, to that seen in the mullet *Mugil cephalus* exposed to the water-soluble fraction of No. 2 fuel oil (Thomas *et al.*, 1980). Plasma glucose concentrations generally return to control levels within 4 d following acute forms of stress (Pickering *et al.*, 1982). Persistent elevations have been recorded, however, in some teleosts exposed to pollutants (Holmberg *et al.*, 1972; Grant and Mehrle, 1973).

Blood lactate can be elevated by stress or muscular activity which often accompanies stress (Love, 1980). Lactate elevation in benzene-exposed individuals preceded the glucose response and a secondary rise was observed by 21 d exposure to 1.0 ppm. Both periods co-occurred with moderately increased swimming activity and restless behavior, indicating that increased lactate was a result of hyperactivity and excitement. Prolonged blood lactate elevations following stress or exercise have been attributed to a catecholamine-mediated slow release from muscle (Wardle, 1978). In the present study, prolonged lactate elevations occurred, and were correlated with the depression of blood pH. A similar relationship has been observed in other fishes (Black, 1958; Cliff and Thurman, 1984). In some investigations, lower blood pH preceded increased lactate (Black *et al.*, 1959; Wood *et al.*, 1977) and has been attributed to respiratory acidosis. Delayed or prolonged decreases in blood pH are the result of metabolic acidosis (Wood *et al.*, 1983). The time-course of blood lactate levels and the correlation between lactate and pH observed in benzene-exposed striped bass suggest metabolic acidosis as a contributory source of increased H^+. The less than one-to-one stoichiometric relationship between lactate and hydrogen ions could be due to increased ventilation as a compensatory mechanism buffering the fall of pH (Hughes, 1981). Greater frequency and amplitude of opercular movement were observed in the present study in fish exposed to benzene during the first 4 d and after 7 d, corresponding to periods of high lactate and low pH. Increased oxygen consumption has been demonstrated in striped bass exposed to benzene (Brocksen and Bailey, 1973).

It is generally believed that mobilization of protein, promoted by corticosteroids, occurs in the form of free amino acids from peripheral tissues for gluconeogenesis and enzyme synthesis in liver (Bondy, 1980). Other studies of teleosts subjected to stressors have demonstrated increased protein levels in plasma and serum (Holmberg *et al.*, 1972; DiMichele and Taylor, 1978; Peters *et al.*, 1980; Brown *et al.*, 1984). Bouck (1972) suggested that protein was released into blood from tissues in the rock bass *Ambloplites rupestus* under hypoxic stress, and Silber and Porter (1953) demonstrated increased plasma albumin in rats treated with cortisone. Results of the present study indicate that benzene-induced stress activates protein metabolic sequences. Elevated levels of serum protein preceded increased plasma glucose in individuals exposed to 1.0 ppm benzene, supporting the contention of Walton and Cowey (1982) that fish utilize protein preferentially to glucose for energy production.

Stress promotes alterations in lipid metabolism and mobilization, although results of research with fish have not revealed a clear, consistent process (Love, 1980; Mazeaud and Mazeaud, 1981). The rapid increase and subsequent return to control values of plasma triglyceride in benzene-exposed striped bass is very similar to the response of blood free fatty acids in carp (*Cyprinus carpio*) following a single administration of epinephrine (Mazeaud *et al.*, 1977). This similarity indicates again that benzene exposure produces a transitory, acute alarm reaction to the perception of a noxious agent and does not evoke chronic GAS-type effects. Increased concentrations of plasma triglyceride have been reported in eels (*Anguilla anguilla*) and coho salmon (*Oncorhynchus kisutch*) exposed to 0.1 ppm pentachlorophenol (Hanes *et al.*, 1968; Holmberg *et al.*, 1972) and in mullet (*Mugil cephalus*) exposed to dibenzofuran (Thomas *et al.*, 1981). Unaltered, or decreased, triglyceride levels were found in mullet exposed to fluorene, carbazole, and dibenzothiophene, however. It appears that the variability of a triglyceride response is dependent on various factors, including the type of stressor and the relative importance of lipid as an energy source in a particular species. Lipid is a major fuel in striped bass, as reflected by relatively high concentrations in blood (1.5 to 3.5 g dl^{-1}), liver (5 to 20%) and muscle (2 to 9%) (Gadbois and Maney, 1983; MacFarlane, unpublished data).

In summary, exposure to benzene concentrations which produced tissue burdens equal to or greater than those found in striped bass in the San Francisco Bay-Delta estuary produced alterations in blood biomolecules characteristic of the GAS. Primary and secondary responses revealed a consistent pattern typical of mild, acute stress. These results indicated that striped bass responded to the sensory perception of a noxious stimulus by activation of the hypothalamo-pituitary-interrenal axis, and that the resistance, or adaptation, stage of the GAS was attained.

Acknowledgements. We thank J. Whipple, M. Bowers, B. Jarvis, S. Moreland, K. Taberski, C. Reilly and other members of the Physiological Ecology Investigation for their excellent technical assistance, and J. Greeley for manuscript preparation and editing. This study was funded by the NOAA Office of Marine Pollution Assessment, allocated under the Marine Protection, Research and Sanctuaries Act, Title II, Section 202, P.L. 95-532. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

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Date of final manuscript acceptance: April 15, 1986.
Communicated by R. S. Carney, Moss Landing