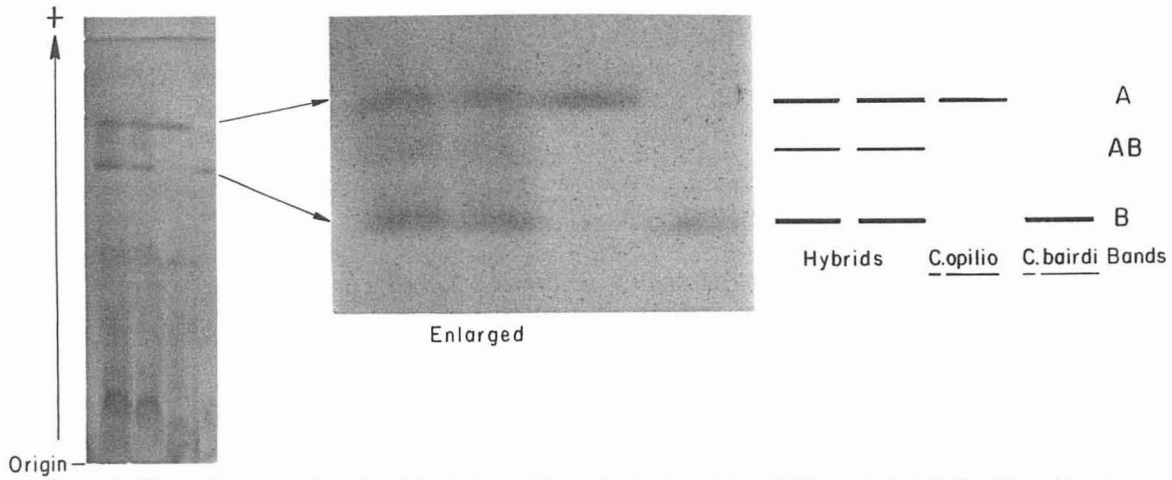

FIGURE 1.—Electropherogram of starch gel showing general muscle protein patterns of *Chionoecetes bairdi*, *C. opilio*, and hybrids.

muscle tissue from 10 *C. bairdi*, 5 hybrids, and 10 *C. opilio* were examined electrophoretically using the methods of Johnson et al. (1972) and the buffer system of Ridgway et al. (1970).

The electrophoretic patterns of general muscle proteins are shown in Figure 1. All *C. opilio* patterns possessed a single band (A), while all *C. bairdi* showed a slower anodally migrating band (B). The five hybrids possessed three bands: A, B, and an intermediate band AB which indicates hybridization between *C. bairdi* and *C. opilio*.

The intermediate band (AB) was less intense than either of the other bands (A or B). A 1:2:1 ratio is expected in random combination of dimeric protein. I thus assume that there is non-random association between the protein units.

Further investigation is needed to determine if the electrophoretic patterns reported here are evident in all possible crosses between the two parent species and that the parental patterns are invariant throughout their ranges.

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EFFECTS OF BENZENE ON GROWTH, FAT CONTENT, AND CALORIC CONTENT OF STRIPED BASS, *MORONE SAXATILIS*

The San Francisco Bay area is a major terminus and refinery area for crude oil, and oil-related activities in the area are expected to increase because of the Alaska pipeline and expanded drilling on the outer continental shelves of California and Alaska. The San Francisco Bay-delta region supports a number of fisheries, including the most important recreational striped bass, *Morone saxatilis*, fishery on the west coast. Information on the toxicity of aromatics in crude oil to striped bass and other fisheries is needed.

The aromatic hydrocarbon, benzene, is one of the major water-soluble components of crude oil. Anderson et al. (1974) reported 6.75 and 3.36‰ in the water-soluble fractions of south Louisiana and Kuwait crude oil standards respectively. In addition to being relatively soluble in water (1,780 ‰ – McAuliffe 1966), benzene is one of the most toxic components of petroleum.

The acute 96-h, TL-50 lethal level (10-11 μl/liter) of constant benzene exposure for juvenile striped bass was determined previously at our laboratory by Meyerhoff (1975). The objective of experiments described here was to see if sublethal levels of benzene, although not inducing death, would inhibit efficient energy utilization by the fish as measured by growth (wet weight, dry weight), fat content, and caloric content. Because the experimental period of 4 wk was relatively short, the juvenile striped bass were exposed to mean high-sublethal concentrations (3.5 μl/liter, SD 1.4; 6.0 μl/liter, SD 1.6) to determine the effects of benzene on growth.

Methods

Juvenile striped bass (mean standard length 18.1 cm, SD 2.3; mean total wet weight 3.39 g, SD 1.1) were obtained from the Tracy pumping plant operated by the Bureau of Reclamation, Tracy, Calif. After being transported by truck to our facility (Korn 1975) the fish were changed to saline water (26‰) during a 3-day period. Juvenile fish occur naturally at this salinity as well as in fresh water. The fish were acclimated for 2 wk to test conditions (salinity 26‰, temperature 15°-16°C, pH 7.8). Thirty-five fish were then placed into each of nine 80-liter fiber glass aquariums and acclimated for one more week. Halver's diet (1957) in pelleted form (5.350 kcal/g) was fed at the rate of 3% of fish body weight per day.

Benzene concentrations were maintained in three aquariums at 3.5 μl/liter benzene and in three at 6 μl/liter benzene; three others served as controls (0 μl/liter). Relatively constant benzene concentrations were maintained using the method of Benville and Korn (1974). The input of benzene-saturated air was balanced by a continuous 2 liters/min water flow through the aquariums.

Benzene concentrations were monitored daily using the gas chromatograph procedure of Benville and Korn (1974). Water quality conditions during the test were as follows: temperature, 15.2°-16.4°C; oxygen, 7.5-7.9 mg/liter; salinity, 25-26‰; pH, 7.7-7.8; ammonia, <0.5 mg/liter.

Seven fish were sampled from each aquarium at 0, 7, 14, 21, and 28 days. The animals were anesthetized with MS-222,[1] killed by severing the spinal cord, blotted dry, weighed individually, dried in a 70°C oven for 4 days, cooled in a desiccator, and reweighed. Three of the fish were then processed for caloric analyses and four for fat analyses.

Calorimetric content was analyzed by individually processing three fish in a Parr adiabatic calorimeter, model 1241.

For fat analyses, the four dried fish were blended with 150-ml MF Freon (monoflourotrichloromethane) in a high-speed blender. The mixture was poured and rinsed into a Buchner vacuum filter through No. 1 filter paper. The filtrate was put into preweighed beakers and evaporated in a hood to dryness. After reweighing the beakers, fat content was calculated.

Data were analyzed with an analysis of variance for factorial design program (BMD 02V–Dixon 1973). The independent factors of tank, week, concentration, and their interactions were tested for significance of effect on the dependent variables of wet weight, dry weight, fat content, and caloric value. Duncan's new multiple-range test (Duncan 1955; Pachares 1959) was used to determine the significant differences between means of levels for treatments found significant in the analysis of variance.

Results

Benzene concentrations varied because of fluctuations in water flow caused by particulate material clogging the valves. The high-level treatment varied from 3.6 to 8.1 μl/liter during the 4-wk test; the low-level treatment varied from 1.5 to 5.4 μl/liter. Analysis of variance of the benzene water concentration showed a significant ($P<0.01$) increase at both levels over the test period. However, the means of low (3.5 μl/liter, SD 1.4) and high (6.0 μl/liter, SD 1.6) concentrations were significantly different ($P<0.01$).

The start of benzene exposure caused pronounced hyperactivity at the high level and a moderate effect at the low level. The fish reacted by attempting to jump out of the water. Fish exposed to the high level attempted to feed but were unable to locate and consume their ration. Random jerking movements were observed when

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

food was introduced. Fish exposed to the low level had some success locating the food, and approximately 50% of the pellets were consumed. Control fish successfully consumed all of their ration within 5 min.

After 1 wk, feeding success on low- and high-dose fish started improving gradually. At the end of the study, the control and low-level fish fed normally, while the high-level fish consumed 50% of their ration.

Analyses of variance of wet weight, dry weight, kilocalories per gram ash-free dry weight, and percent fat between concentrations, weeks, and tanks yielded the following results (Tables 1, 2). There was a significant decrease in wet weight ($P<0.05$), dry weight ($P<0.01$), and percent fat ($P<0.01$) with increasing concentration (Table 2). Concentration levels varied significantly ($P<0.05$) (Table 1): Wet weight was less at 6.0 μl/liter than controls and did not vary significantly between 3.5 μl/liter and controls or between 3.5 and 6.0 μl/liter. Dry weight was less at 6.0 μl/liter than at 3.5 μl/liter and controls but did not vary significantly between controls and 3.5 μl/liter. Percent fat was less at 6.0 and 3.5 μl/liter than in controls. There was no significant difference in percent fat between 6.0 and 3.5 μl/liter.

There was a significant increase in dry weight ($P<0.05$) during the last week at all exposures (Table 2, Figure 1). There was no significant difference between treatments in kilocalories per gram ash-free dry weight (Table 2). The significant interaction between concentration and tank ($P<0.05$–Table 2) is a result of experimental design in which certain tanks were always at a high or low concentration. No significant variation occurred between tanks.

TABLE 1.–Mean wet wet and dry weights and fat caloric content of one control and two test groups of striped bass, *Morone saxatilis*, exposed to benzene for 4 wk.

Treatment mean concentration (μ l/liter)	Variable[2]			
	Wet weight (g)	Dry weight (g)	Fat (%)	Ash-free dry weight (kcal/g)
Control	2.7135	0.8721	39.2	6.8123
Low level (3.5)	2.6062	0.8137	34.1	6.8435
High level (6.0)	2.3951	0.7242	32.2	6.7451
Total number of fish	315	315	45	45

[1]The three treatments used three replicate tanks per treatment sampled at 0, 1, 2, 3, and 4 wk. Tests for wet and dry weights had seven fish/tank per week; tests for percent fat had four fish/tank per week; and tests for kilocalories/gram ash-free dry weight had three fish/tank per week.

[2]Duncan's new multiple-range test of differences between means of treatment levels was performed. Means grouped above with same bar are not significantly different at the 5% level. Means not grouped with same bar are significantly different at the 5% level (Duncan 1955; Pachares 1959).

Discussion

Although acclimated, fish in all treatments were stressed from crowding and insufficient water movement. This was unavoidable because space and equipment were limited. Consequently, the control fish did not grow at the same rate as similar fish held in larger tanks at this facility. In spite of these limitations, significant relative changes in growth rate and fat content did occur between exposure treatments. Wet weight, dry weight, and fat content decreased with increasing concentration as expected. This was probably due

TABLE 2.–Analysis of variance of treatment effects of benzene concentration (μl/liter), week, and tank number on wet weight (g), dry weight (g), kilocalories per gram ash-free dry weight, and percent fat of juvenile striped bass, *Morone saxatilis*.

Dependent variable and source of variation	df	Sum of squares	Mean square	F ratio	Probability
Wet weight:					
Concentration	2	5.511	2.756	3.16	$P< 0.05$
Week	4	7.750	1.938	2.20	NS
Tank	2	3.050	1.525	1.75	NS
Concentration-week	8	11.673	0.909	1.04	NS
Concentration-tank	4	11.255	2.814	3.22	$P< 0.05$
Week-tank	8	11.673	1.459	1.67	NS
Concentration-week-tank	16	15.516	0.970	1.11	NS
Within (error)	270	235.675	0.873	—	—
Total	314	302.103	—	—	—
Dry weight:					
Concentration	2	1.165	0.583	5.40	$P< 0.01$
Week	4	1.232	0.308	2.85	$P< 0.05$
Tank	2	0.420	0.210	1.94	NS
Concentration-week	8	0.933	0.117	1.08	NS
Concentration-tank	4	1.214	0.304	2.81	$P< 0.05$
Week-tank	8	1.367	0.171	1.58	NS
Concentration-week-tank	16	2.166	0.135	1.25	NS
Within (error)	270	29.137	0.108	—	—
Total	314	37.634	—	—	—
Kilocalories per gram ash-free dry weight:					
Concentration	2	0.076	0.038	0.50	NS
Week	4	0.404	0.101	1.33	NS
Tank	2	0.284	0.142	1.87	NS
Concentration-week	8	1.177	0.147	1.93	NS
Concentration-tank	4	0.147	0.037	0.49	NS
Week-tank	8	0.667	0.083	1.09	NS
Residual (error)	16	1.222	0.076	—	—
Total	44	3.977	—	—	—
Percent fat:					
Concentration	2	383.902	191.951	13.42	$P< 0.01$
Week	4	99.539	24.885	1.74	NS
Tank	2	52.878	26.439	1.85	NS
Concentration-week	8	138.843	17.355	1.21	NS
Concentration-tank	4	55.000	13.750	0.96	NS
Week-tank	8	190.733	23.842	1.67	NS
Residual (error)	16	228.929	14.308	—	—
Total	44	1,149.824	—	—	—

NS = not significant.

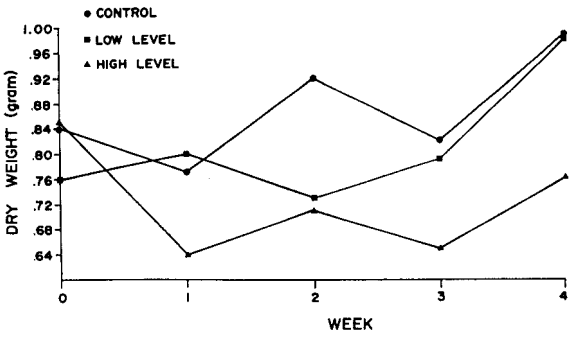

FIGURE 1.—Average weight of each of three groups of striped bass, *Morone saxatilis*, exposed to three concentrations of benzene (0, 3.5, and 6.0 µl/liter) for 4 wk. The dry weight of high-level exposure fish was significantly less ($P<0.01$) than the other two groups at the end of the first week and thereafter. The dry weight of the three groups combined was significantly higher ($P<0.05$) than in previous weeks.

mostly to impaired food localization at higher concentrations. A similar effect on the nervous system is documented by Brocksen and Bailey (1973). The energy required to metabolize benzene could also decrease efficient utilization of energy for growth and fat deposition.

There was an apparent acclimation of the fish to benzene at the low level (3.5 µl/liter) by the end of the 4-wk exposure, as reflected by the dry weight of the fish (Figure 1). After 4 wk at high level (6.0 µl/liter), fish also appeared to begin to recover from effects. This was substantiated by observations of improved feeding response in exposed fish as the experiment progressed. Nevertheless, definite effects of benzene on growth parameters were noted at 6.0- and 3.5-µl/liter levels of benzene. Although the fish may be able to adapt by metabolic detoxification and depuration of benzene and metabolites, after more prolonged periods the competitive effects on energy utilization may not only decrease growth but also increase mortality or reduce ability to withstand environmental stress.

The parameters measured in this study show effects at the low µl/liter levels. In most situations, it is unlikely that fish would be exposed to benzene above the nl/liter level except shortly after catastrophic spills. Anderson et al. (1974) obtained a concentration of several µl/liter benzene in water-soluble extracts of crude oils. In the marine environment, dilution and volatilization of benzene would probably lower the concentration of benzene rapidly. Research on effects at the nl/liter level is needed along with monitoring information on actual concentrations of benzene in chronically polluted environments. Such situations may induce a reduction in growth rate and fat deposition which would have implications in the reproductive potential of exposed species. Studies of chronic effects of low concentrations of benzene on reproduction, including fecundity, egg size, embryonic development, and larval survival, are indicated. Some of these studies have been completed at the Tiburon Laboratory and will be reported on later.

Acknowledgments

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FERTILIZATION METHOD QUANTIFYING GAMETE CONCENTRATIONS AND MAXIMIZING LARVAE PRODUCTION IN *CRASSOSTREA GIGAS*

Most workers obtain oyster larvae by using experimental methods similar to those reported by Galtsoff (1964). Although useful in most hatchery or laboratory investigations, these methods do not quantify gamete concentrations. To obtain specific larval concentrations, most researchers dilute dense postfertilization concentrations.

This paper reports on a method of estimating sperm concentrations of Pacific oyster, *Crassostrea gigas*, using colorimetric techniques, and on a method of fertilization using small volumes of seawater and known gamete concentrations. We also present an index which may be useful in evaluating the efficiency of fertilization. These methods were developed during 1973 and should prove useful in the study and production of cultured oysters.

Materials and Methods

Pacific oysters were obtained from Fowler Oyster Co. on Yaquina Bay, Newport, Oreg. Sand-filtered seawater of 25-32‰ salinity and pH 7.0-8.1 was collected at the Oregon State University Marine Science Center (MSC) at Newport, exposed to ultraviolet light (3.785 liters/min), diluted (when necessary) to 25‰ with distilled water, and stored in Nalgene carboys. This salinity is within the range recommended for *C. virginica* by Davis and Calabrese (1964), and was used for maintenance of oysters and for experiments on fertilization and early larval development. In laboratory procedures, all glassware was initially acid-washed; used glassware was carefully cleaned and rinsed several times first in tap water and then in distilled water; all polyethylene tubing was Tygon[1] R3606 (nontoxic by bioassay, Breese, MSC, unpubl. data); gametes and larvae were confined in glass containers only (except for momentary exposure to stainless steel syringe needles and nylon screen); all seawater used in fertilization experiments was Millipore-filtered (0.47 μm) and stored in glass screw-cap bottles with Parafilm-lined caps (nontoxic by bioassay, Breese unpubl. data).

Procurement of Gametes

To enhance gonad development, we conditioned mature oysters in seawater at 16.0° ± 1.0°C for 3-6 wk (Loosanoff and Davis 1963). To identify test oysters, we drilled a 0.8-mm (1/32-inch) hole in the umbo and attached a 6.4- × 15.9-mm numbered plastic tag (Howitt Plastics Co., Mollala, Oreg.) with monofilament. After conditioning, access to the gonads was made by drilling a 1.2-mm (3/64-inch) hole in the posterodorsal region of the right valve. We extracted gametes with a 2.5-cm^3 glass syringe fitted with a 20-gauge 38-mm needle containing about 0.5 ml of seawater (Lannan 1971). Oysters containing either intensively motile sperm or eggs greater than or equal to 36 μm were kept for fertilization experiments. To prevent spawning after extractions, we isolated individual oysters for 12-24 h in 3-liter beakers containing seawater at 12°C.

Prior to gamete extraction we raised the temperature of all donor oysters to 27.0° ± 0.5°C, a temperature within the range recommended by Davis and Calabrese (1964) for fertilization and larval development. Oysters were transferred from the conditioning tray to an 18.9-liter (5-gallon) tank containing 11.4 liters (3 gallons) of seawater at 16.0° ± 1.0°C; a 100-W aquarium heater

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.