Seasonal and Laboratory Variations in the Health of Grass Shrimp *Palaemonetes pugio:* Dodecyl Sodium Sulfate Bioassay

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INTRODUCTION

Examination of the effects of pollutants on aquatic ecosystems usually involves standard toxicity testing on organisms collected from a local area. It is relatively simple to control and monitor many of the variables present in toxicity experiments such as toxicant concentrations and the water quality parameters of dissolved oxygen, temperature, and salinity or hardness. However, a crucial parameter, the health of the experimental organisms, is difficult to determine and measure accurately.

Review publications (SPRAGUE, 1969; WALDICHUK, 1973) have stressed the importance of using healthy test organisms for toxicity experiments. Some of the problems involved in holding marine organisms in the laboratory for toxicity tests were discussed by PERKINS (1972). A recent paper on bioassay methods by STEPHAN (1975) recommended research directed toward determining the "healthiness" of test organisms.

Various methods have been proposed to determine the health of test organisms. Many workers in the past have observed collected animals for one or two weeks, discarding groups exhibiting greater than 10% mortality (A.P.H.A., 1971) during the holding period. HANSEN *et al.* (1974) acclimated test organisms to laboratory conditions for seven days and did not use groups showing greater than 1% mortality or abnormal behavior in a 48-hour period before testing.

Another approach toward standardization of toxicity test results is to use standard test organisms. The brine shrimp, *Artemia*, may be stored indefinitely as dry eggs and are ideal for toxicity testing according to ZILLIOUX et al. (1973). However, standard organisms are not necessarily healthy and applicability to natural populations is reduced. Also, organisms which are easily hatched or maintained in the laboratory have not been stressed by natural conditions and are usually hardy, adaptable animals which produce high median lethal toxicity (TLm) values.

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Thus, it has been suggested (LAROCHE et al., 1970; WILSON et al., 1973) that workers use a standard reference toxicant prior to toxicity experiments with a given group or collection of organisms. LAROCHE et al. (1970) advocated the use of dodecyl sodium sulfate (DSS) as a standard toxicant while WILSON et al. (1973) recommended the use of a reagent grade hydrocarbon. ANDERSON et al. (1974) tested successive collections of estuarine fish and crustaceans with DSS as a measure of organism health.

The reported study involved numerous static toxicity tests with DSS on groups of grass shrimp, primarily Palaemonetes pugio (Holthuis), collected from June 1972 through April 1974 from the same area of Galveston Bay, Texas. The purpose of this work was to determine the seasonal variation in the resistance of these crustaceans to DSS and the response of the organisms to DSS after time in the laboratory. This work was conducted on various collections of shrimp prior to their subsequent use in toxicity and physiological experiments with petroleum hydrocarbons.

Materials and Methods

Organisms were collected by seine from Spartina marsh areas at 8 Mile Road, Galveston, Texas. The water temperature varied seasonally from approximately 14° to 32°C. The salinity varied between 18 and 26 parts per thousand (o/oo). Animals were transported to the laboratory in styrofoam chests containing aerated natural sea water. They were held at 20° ± 2°C in charcoal filtered artificial sea water prepared with distilled water and Instant Ocean (Aquarium Systems, Inc., Eastlake, Ohio) to a salinity of 15 o/oo. New collections were exposed to different concentrations of DSS within five days of their arrival in the laboratory, usually after 48-72-hour acclimation. Some groups were retested after being in the laboratory for longer periods. All experiments were conducted at 15 o/oo and 20° ± 1°C, and only non-gravid adults were used.

Initial tests used 1.0-ℓ chambers containing 500 ml of test solution, five replicates per concentration, and two organisms per chamber. Later experiments were conducted in 3.0-ℓ chambers containing 2000 ml of test solution, two replicates per concentration, and five organisms per chamber. Gentle aeration was provided to each chamber during experiments.

DSS is a synthetic anionic detergent of the linear alkylate sulphonate type (ABEL, 1974). The DSS used in the present work was a laboratory grade reagent (Fisher Scientific Co.) and contained a linear hydrocarbon chain of 12 carbon atoms.

All DSS bioassays were conducted similarly. The two variables tested related to the time of year of organism collection and the length of time that the animals were held in the laboratory prior to testing. Results are presented as 48- and 96-hour TLm values in ppm DSS added by weight. TLm values were computed by graphical analysis according to A.P.H.A. (1971).

Toxicant concentrations were prepared by adding 1.0 g of DSS powder to 2000 ml of 15-o/oo artificial sea water. This stock solution contained approximately .0005 g DSS/ml such that 1.0 ml of stock equalled 1 ppm DSS in a total volume of 500 ml. Stock solutions were freshly prepared the day that a test was to begin. Animals were fed Tetramin fish food daily during the holding period but not during the 96-hour tests. Test chambers were checked at least twice a day and dead organisms were removed upon discovery.

Results

Results of the tests are presented in Tables 1 and 2. Table 1 shows the TLm values determined for eleven separate grass shrimp collections from the same area. Grass shrimp were shown to be relatively resistant to DSS, especially

TABLE

Table 1. Collection data and 48- and 96-hour TLm values for groups of Palaemonetes exposed to DSS.

Collection date	48-hour TLm, ppm	96-hour TLm, ppm
June 1972	137.5	126.5
July 1972	160.0	140.0
October 1972	112.5	112.5
January 1973	77.0	69.0
March 1973	>160.0	160.0
April 1973	162.0	162.0
October 1973	112.0	<112.0
December 1973	90.0	72.0
January 1974	70.0	<70.0
February 1974	>90.0	90.0
April 1974	115.0	98.0

during the spring and summer months. The highest 96-hour TLm value was 162.0 ppm for April 1973 while the lowest value was 69.0 ppm for January 1973. Similar TLm values of 112 ppm were found for October 1972 and 1973. The toxic action of the DSS solution was greatest during the initial 48 hours of the tests as shown by the similarity of the 48- and 96-hour values.

The effect of maintaining the shrimp in the laboratory for different lengths of time is shown in Table 2. The data demonstrate that in every case organisms maintained in the laboratory for longer periods were more susceptible to the standard toxicant. A holding period of two weeks was enough to significantly decrease the TLm values in comparison with freshly collected animals. One experiment with three different groups of grass shrimp determined the wet weights of individual shrimp. There were definite size differences yet the time in the laboratory was apparently more important in lowering DSS resistance than size.

TABLE

Table 2. The 48- and 96-hour TLm values in ppm DSS for separate groups of shrimp held in the laboratory for different time periods.

Month of Collection	Time in Lab	Average Wet Wt.	48-hr TLm, ppm	96-hr TLm, ppm
December 1973	<1 week	-	90.0	72.0
December 1973	>1 month	0.58 gm	66.0	52.0
February 1974	2 days	0.150 gm	>90.0	90.0
February 1974	2 weeks	0.307 gm	75.0	52.5
April 1974	5 days	-	115.0	98.0
March 1974	1 month	-	72.0	55.0

DISCUSSION

The data indicate that grass shrimp tolerated substantial concentrations of DSS in comparison to marine fish and crustaceans. A review of detergent toxicity to fish by ABEL (1974) reported toxic concentrations between 0.4-40.0 ppm. Yet NAGELL et al. (1975) found higher TLm values in tests with oil dispersants, which are basically detergents, and species of fish, bivalves and crustaceans. His TLm values were as high as 1000 ppm; however, both water-base

dispersants and oil-base dispersants were tested with the oil-base being found more toxic.

Generally, smaller crustacean species have been found more sensitive to DSS and detergents than larger crustaceans such as Palaemonetes and Crangon, a related European shrimp. Toxicity experiments with the small opossum shrimp, Mysidopsis (ANDERSON et al., 1974) showed 2.0 ppm DSS to be toxic in 24 hours and ZILLIOUX et al. (1973) found a 48-hour TLm of 3.0-4.0 ppm DSS for the brine shrimp Artemia. These low values are in contrast to the data of SWEDMARK et al. (1971) and PORTMANN (1972) who determined that the shrimp, Crangon, was relatively resistant to detergents. They found TLm values around 100 ppm which agree with our data on Palaemonetes. Estuarine fish, Fundulus heteroclitus, exposed to DSS exhibited 48- and 96-hour TLm values of 5.6 ppm (LAROCHE et al., 1970).

The interesting aspects of our data were that Palaemonetes tolerated relatively high levels of DSS especially when collected in the spring and summer months and that they show significant decreases in resistance to the standard toxicant with time in the laboratory, even while appearing normal and healthy. A number of explanations can be suggested.

Many studies have found Palaemonetes most abundant in the summer. Reproductive activity begins in April and young shrimp mature in a few months over the summer (WOOD, 1967; KNOWLTON and WILLIAMS, 1970; WELSH, 1973). KNOWLTON and WILLIAMS (1970) have reported that growth of adult grass shrimp largely ceases during the winter months. Our Palaemonetes collections during winter months produced fewer specimens of smaller size with no gravid females. Larger summer and fall populations suggest optimum environmental conditions at these times. Omnivorous Palaemonetes feed on algae associated with detritus as well as smaller crustaceans. During winter months, lower temperatures and possibly decreased food availability may reduce the nutritive status of these shrimp making them more susceptible to DSS.

The data in Table 2 demonstrated that organism health declined with increasing time in the laboratory. Although the grass shrimp exhibited 100-% survival and behaved in a normal fashion during a holding period of months they were less resistant to DSS after being held only two weeks. Estuarine areas are constantly fluctuating in temperature, salinity, and photoperiod while the laboratory conditions are stable. However, it is more likely that the somewhat

crowded conditions and amounts and types of food available were the primary factors in the lower TLm values found for shrimp held in the laboratory. Shrimp tested after one month in the laboratory were older than animals tested initially. However, since grass shrimp live for at least one year in nature it is likely that the laboratory conditions caused the increased susceptibility to DSS rather than the fact that the organisms were 30-days older. Shrimp exhibited no obvious diseases during holding.

Temperature relates directly to the activity levels of organisms and usually has an affect on the toxicity of various pollutants. Higher temperatures increased the toxicity of petroleum hydrocarbons (COX, 1974). SWEDMARK et al. (1973) have emphasized the importance of activity levels, which are temperature related, on detergent toxicity. Temperature effects on DSS toxicity were demonstrated by the authors when shrimp from an April collection died in 24 hours at 30 ppm DSS when laboratory water temperatures inadvertently rose from 20° to 29°C. The present DSS toxicity work was carried out at 20°C. This temperature was higher than the environmental water temperatures in winter but lower than summer temperatures. Thus the organisms collected during the winter months were naturally acclimated to lower temperatures and when tested at 20°C revealed lower resistance to DSS than summer collections. Environmental temperatures in March and April are close to the test temperature which may be one reason for the high TLm values determined for animals collected in these months.

Grass shrimp were less susceptible to DSS than estuarine fish or smaller crustaceans. It is the nature of surface-active agents to collect at surfaces and this process is enhanced by aeration. Hence organisms which prefer to rest quietly at the bottom of bioassay containers such as Palaemonetes would be expected to be able to endure higher detergent toxicity levels than fish or smaller crustaceans which continually swim in the water column during tests.

The toxic mode of action of the DSS molecules may also be linked to the activity of the experimental organisms and the resulting amount of exposure of critical cellular membranes. ABEL (1974) has listed the physical effects of detergents as severe gill damage and destruction of chemoreceptor organs. A 1% solution of DSS, has been observed to cause sudden removal of the cell membrane of Amoeba proteus (GOLDACRE, 1968). Detergents at lower concentrations may alter membrane permeability. Differences in toxicity have been found for the fish Fundulus

and the shrimp Palaemonetes. Increased activity by organisms in the water column, resulting in greater exposure of respiratory membranes, would render more active organisms more susceptible to the DSS.

The results suggest that the best procedures to follow regarding toxicity testing of aquatic organisms would be to begin experiments as soon as possible after collection. Ideally, the testing would be performed under temperature and salinity parameters similar to field conditions so that acclimation times could be as short as possible. For routine bioassays organisms should be held and observed for two days and then tested with a standard toxicant. Further testing of pollutants could begin as soon as it was apparent from the standard toxicant results (48-hour TLm) that a particular collection of organisms was in the normal health range for that species at that time of year. It is well known that bioassay data obtained from field collections of organisms are highly variable. This study has demonstrated a possible cause of this variability and suggests steps that may be taken to reduce or partially account for this variability.

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