Arch. Environ. Contam. Toxicol. 31, 293–295 (1996)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Effects of the Anionic Surfactant, Sodium Dodecyl Sulfate, on Newly-Hatched Blue Crabs, *Callinectes sapidus,* and Other Routinely Tested Estuarine Crustaceans

V. K. Whiting[1], G. M. Cripe[2], J. E. Lepo[1]

[1] Center for Environmental Diagnostics and Bioremediation, University of West Florida, 11000 University Parkway, Pensacola, Florida 32514, USA
[2] U.S. Environmental Protection Agency, National Health Environmental Effects Research Laboratory, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, Florida 32561, USA

Received: 29 October 1995/Revised: March 19, 1996

Abstract. This study describes the use of newly hatched larvae of *Callinectes sapidus* (blue crab) in a 48-h acute toxicity test and compares their sensitivity to two other estuarine crustaceans (*Mysidopsis bahia* and *Palaemonetes pugio*) commonly used for evaluation of effects of potentially toxic materials. *C. sapidus* larvae were twice as sensitive to sodium dodecyl sulfate as ≤ 24-h post-release *M. bahia,* and five times more sensitive than ≤ 24-h-old *P. pugio* larvae. We found the blue crab toxicity test to be simple, rapid and accurate and it provides low variability and high reproducibility. Since the data indicate high sensitivity of this commercially important species to a reference toxicant and the potential impact on its survival during a critically sensitive developmental stage, we propose future research further evaluating *C. sapidus* as a potential toxicity test species.

Blue crabs, *Callinectes sapidus,* have been the subject of extensive life history and reproduction studies, as well as investigations of migrational patterns (Perry and Van Engel 1979; Steele and Perry 1990). They inhabit both upper estuarine and offshore waters at various life stages. Records of their life cycle indicate that larvae travel from onshore ocean areas through estuary mouths to the upper reaches of the estuary where they encounter low salinity as they grow, mature and mate. Gravid females move to higher salinity areas, usually at the mouth of estuaries, where larvae are broadcast to onshore currents and swept out to sea to develop to the megalops stage (Costlow and Bookhout 1959). Consequently, as both an estuarine and a coastal species (Sulkin 1974), they have the potential to encounter an extensive variety of pollutants.

The blue crab is a commercially important species in the Western Atlantic and Gulf of Mexico. In 1994, the U.S. hardshell blue crab fishery of 209 million pounds was valued at $137 million (Holliday and O'Bannon 1994).

Because of the life history patterns and short life span (1–2 years), blue crab landings are variable year-to-year and subject to crash-boom population patterns. Therefore, this species merits examination of its sensitivity to environmental pollutants, and in recent years, larvae have been exposed to several toxicants (Bookhout and Costlow 1975; Bookhout et al. 1979, 1980, 1984; McKenney and Costlow 1981). Because of size and culturing requirements, the estuarine crustaceans most often used for laboratory evaluation of toxic material are mysids (*Mysidopsis bahia*), and grass shrimp (*Palaemonetes pugio;* ASTM 1988). Whereas these species are easily cultured and maintained in the laboratory, their sensitivities may not parallel those of commercially important species such as blue crabs. The results from exposures of blue crabs and routinely tested estuarine crustaceans exposed to the same toxic materials would facilitate an evaluation of protection of this species with data from surrogate test organisms.

In addition to species differences, life stage can be a significant factor in sensitivity of crustaceans. Several studies have suggested that juvenile and larval forms of crustaceans are more sensitive to toxicants than their adult forms (Nimmo *et al.* 1971; Conner 1972; Armstrong *et al.* 1976; Cripe 1994). Toxicity studies with early life stages of blue crabs examined the effects of chemicals on larval development through metamorphosis to first crab stage in 30- to 40-day exposures (Bookhout and Costlow 1975; Bookhout *et al.* 1979, 1980, 1984).

This study was conducted to extend the information base on an important economic species, to develop an exposure technique with newly released blue crab larvae, and to compare their sensitivity to that of mysids and grass shrimp. The toxicant was sodium dodecyl sulfate (SDS), a commonly used reference toxicant.

Contribution No. 943 of the U.S. Environmental Protection Agency, Health Environmental Effects Research Laboratory, Gulf Ecology Devision, Gulf Breeze, FL

Correspondence to: G. M. Cripe

Materials and Methods

Test Organisms

All bioassays were conducted with test organisms that were at the earliest life stage feasible. To meet the criterion for the blue crab tests, females blue crabs were collected near the mouth of Pensacola Bay, Florida, in the fall of 1993 at 26°C and 26 parts per thousand (‰) salinity. Females with undeveloped eggs, or "orange sponge," were chosen in order to observe embryological development and to avoid fungal or bacterial infections. The females were held in 25 to 26‰ seawater until just prior to hatching of larvae. Samples of the egg mass were taken from each female and microscopically examined to determine the health and developmental stage of the embryos, as described by Costlow and Bookhout (1959). Other egg samples were incubated in 26‰ seawater in tissue culture cell wells at 25°C for use in estimating the hatching time for eggs remaining on the females.

Female crabs were tranferred to a recirculating spawning tank adapted from the design by Middaugh and Hemmer (1984). Seawater of 26‰ was filtered to 0.45 μm, heated to 25°C, and recirculated from a single holding tank with a 150 μm mesh nylon screen at the outflow through a biological filter. When eye pigment developed, the egg mass appeared black, and embryo heartbeats were observed prior to hatching. The resulting larvae were collected in the nylon screen and washed several times in filtered (0.45 μm) 26‰ seawater. The positively phototactic response of the larvae was used to separate them into groups of approximately 100 for distribution among exposure containers.

Grass shrimp were collected in Pensacola Bay, Florida, in March, 1994 at 17°C and 5‰ and acclimated to 25°C and 20‰ within 24 h of collection. Whenever young were needed for testing, a group of ten gravid females, with eggs at various stages of maturity, were placed in an aerated 30 gallon aquarium under static conditions in the dark. The larvae were collected the next day using a light source as an attractant. *M. bahia* (≤ 24-h post-release) were obtained from laboratory cultures maintained at 25°C and 20‰ salinity.

Toxicant Exposure

Sodium dodecyl sulfate (SDS) was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, at 99% purity. It was dissolved in deionized water to produce a stock concentration of 10,000 parts per million (mg/L). The required volume of stock solution was added to 200 ml of seawater of appropriate test salinities (blue crabs, 26‰, mysids and grass shrimp, 20‰) in 250-ml glass culture dishes to obtain the desired test concentration. Blue crab and grass shrimp larvae were exposed to nominal concentrations of 100, 50, 25, 12.5, and 6.25 mg/L SDS. Mysids were exposed to nominal concentrations of 50, 25, 12.5, 6.25, and 3.12 mg/L SDS. Test concentrations for mysid exposures were based on previous tests providing a 48-h LC50 of 12.3 mg/L SDS for 5-day-old mysids.

Biomass per animal differs substantially among species at these early life stages. To maintain comparable oxygen levels in the test conditions, a different number of animals was used in the exposure vessels. Twenty blue crab larvae or ten mysids or grass shrimp were exposed in each of two replicates of each concentration.

Test containers were covered and placed on a rotary shaker table at 50 rpm and maintained at 25 ± 1°C with a 14-h light/10-h dark photoperiod. Mortality, temperature, salinity, pH, and dissolved oxygen were monitored daily. Because of cannibalism, mysids were fed a minimal amount of brine shrimp (*Artemia*) nauplii during the tests. All toxicity tests were terminated after 48 h.

LC50s and 95% confidence intervals were calculated by the binomial method (Stephan 1977). Comparison of LC50s among species was evaluated by Analysis of Variance and Duncan's Multiple Range tests (SAS Institute 1987).

Table 1. 48-h LC50s for larval blue crabs, grass shrimp, and juvenile mysids exposed to sodium dodecyl sulfate

Organism	48-h LC50[a]	Mean LC50[b]
Blue crabs		
Test 1	12 (6.2–25)	9.8
Test 2	8.8 (6.2–12)	
Test 3	8.8 (6.2–12)	
Mysids		
Test 1	17 (12–25)	17
Test 2	17 (12–25)	
Test 3	16 (12–25)	
Grass shrimp		
Test 1	32 (25–50)	34
Test 2	35 (25–50)	
Test 3	34 (25–50)	

[a] Concentrations are in mg/L with confidence intervals in parentheses. LC50s within each species were calculated by the binomial method (Stephen 1977)

[b] Analysis of variance and Duncan's Multiple Range testing of the computed LC50s indicated that all species responded significantly differently ($\alpha = 0.0001$) from each other

Results

The pH of seawater at the test initiation ranged from 8.0 to 8.3. By test termination, pH increased by 1 unit or less in blue crab exposures, 0.4 unit or less in mysid tests, and 0.2 units or less in grass shrimp tests. Dissolved oxygen concentrations in the test vessels were well above ASTM guidelines, averaging 6.4 mg/L (range of 6.1 to 6.6) for blue crab, 6.25 mg/L (range of 4.8 to 7.3) for mysid, and 6.2 mg/L (range of 5.8 to 6.6) for grass shrimp tests. Control animal survival throughout the tests exceeded 95 percent.

All tests were conducted with young from different females, and each test produced similar 48 h LC50s within each species, with the exception of grass shrimp. The grass shrimp LC50s ranged from 32 to 35 mg/L, with a mean of 34 mg/L (Table 1). Newly released blue crab larvae were approximately twice as sensitive as 24-h post-release mysids with an average LC50 of 9.8 mg/L. However, there was considerable overlap in the 95% confidence intervals. Mysids (LC50 of 17 mg/L) were approximately three times as sensitive as grass shrimp larvae, which had a mean LC50 of 48 mg/L (Table 1).

An analysis of variance indicated that the mean LC50s for each species exposed to SDS were significantly different ($\alpha = 0.0001$). In fact, all three species were different from each other (Duncans Multiple Range test, $p = 0.05$).

Discussion

Availability of gravid blue crabs and their location at time of collection limited the test salinity for this species. Blue crab larval tests were conducted at 26‰ salinity, the same as in waters from which females were collected, to eliminate salinity stress of the embryos before testing. This salinity was higher than that used with mysid or grass shrimp tests.

Feeding test animals during static exposures increases the likelihood of degraded water quality, *e.g.,* some short-term tests are adversely affected by holding problems, metabolic buildup, or starvation stresses. Since this exposure lasted 48 h, it was not necessary to feed the grass shrimp and blue crabs. Our preliminary tests demonstrated that newly released blue crab larvae could survive up to 6 days without feeding, and grass shrimp could survive through their first molts without feeding. Thus, only mysids were fed.

Although experiments by other researchers have examined the effects of contaminants on larval blue crabs, few data from the first 48-h post-hatch are available. Most studies examined endpoints beyond a 48-h time frame and up to 50 days after hatch (Bookhout and Costlow 1975; Bookhout *et al.* 1979, 1980, 1984). Also, these tests are labor intensive and time consuming, requiring daily handling and feeding. The present study allows a 48-h static toxicity test with newly released blue crab larvae, with no feeding and very little handling. Blue crab larvae were most sensitive to SDS, followed by mysids, and the least sensitive were grass shrimp (by five times).

The low variability of blue crab test results from different females suggests that this technique provides results with high reproducibility. The results of the mysid and grass shrimp tests were likewise highly reproducible.

Studies of toxic effects on crustaceans have indicated that the earliest life stages are often the most sensitive. Conner (1972) reported one to three orders of magnitude increased sensitivity in larvae of sand shrimp (*Crangon crangon*), green crabs (*Carcinus maenus*) and European lobsters (*Homarus gammerus*) to mercury, copper and zinc as compared to adults. Armstrong *et al.* (1976) documented three orders of magnitude difference between larval and adult stages of Dungeness crabs (*Cancer magister*) to methoxychlor, and Nimmo *et al.* (1971) found larval pink shrimp (*Penaeus duorarum*) to be three to ten times more sensitive than adults to Aroclor® 1254. The data from this study indicate that the early life stage of blue crabs is likewise very sensitive to toxic materials.

Blue crab larvae are generally planktonic, found drifting within the top 2 meters of offshore waters (Van Den Avyle and Fowler 1984; Sulkin and Van Heukelem 1982; Epifanio 1988). The early life stage of this species might be utilized to evaluate the effects of human activities, such as offshore drilling and industrial effluent discharge. Furthermore, they are representative of commercially important species found in open water. Additional use of the blue crab larval toxicity test with a variety of toxicants (*e.g.,* pesticides, heavy metals and organic compounds) would help to evaluate the breadth of sensitivity of this crab to toxic materials. Such studies would also enhance our ability to compare the sensitivity of this test to that using other species.

Acknowledgments. We are grateful to the following individuals for their help in field collections and technical assistance: Katharine Edwards, Joanne Konstantopoulus, and Robert Quarles. We also thank Foster L. Mayer, Jeff Kavanaugh, and Geneva Norton for critical reading of the manuscript and helpful discussions.

This work was supported through cooperative agreement (CR-818998) between the USEPA's Gulf Breeze Environmental Research Laboratory and the University of West Florida.

References

Armstrong DA, Buchanan DV, Mallon MH, Caldwell RS, Millemann RE (1976) Toxicity of the insecticide methoxychlor to the Dungeness Crab *Cancer magister.* Mar Biol 38:239–252

ASTM (1988) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. In: Annual Book of ASTM Standards. Vol. 11.04. Amer. Soc. Test. Mat. Philadelphia, PA, pp 378–397

Bookhout CG, Costlow, Jr. JD (1975) Effects of mirex on the larval development of blue crab. Water Air Soil Pollut 4:113–126

Bookhout CG, Costlow, Jr. JD, Monroe RJ (1979) Effects of methoxychlor on larval development of mud crab and blue crab. Water Air Soil Pollut 5:349–365

——— (1980) Kepone effects on larval development of mud crab and blue crab. Water Air Soil Pollut 13:57–77

Bookhout CG, Monroe RJ, Forward, JR. RB, Costlow, Jr. JD (1984) Effects of hexavalent chromium on development of crabs, *Rhithropanopeus harrisii* and *Callinectes sapidus.* Water Air Soil Pollut 21:199–216

Conner PM (1972) Acute toxicity of heavy metals to some marine larvae. Mar Pollut Bull 3:190–192

Costlow, Jr. JD, Bookhout CG (1959) The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. Biol Bull 116:373–396

Cripe GM (1994) Comparative acute toxicities of several pesticides and metals to *Mysidopsis bahia* and post-larval *Penaeus duorarum.* Environ Toxicol Chem 13:1867–1872

Epifanio CE (1988) Transport of invertebrate larvae between estuaries and the Continental Shelf. Amer Fish Soc Symp 3:104–114

Fisheries of the United States 1994 (1995) Holliday MC, O'Bannon BK (eds) Current Fishery Statistics No. 9300. NOAA, U.S. Department of Commerce

McKenney, Jr. CL, Costlow, Jr. JD (1981) The effects of salinity and mercury on developing megalopae and early crab stages of the blue crab, *Callinectes sapidus* Rathbun. In: Vernberg J, Thurberg F, Calabrese A, Vernberg W (eds), Biological monitoring of marine pollutants. Academic Press NY. pp. 241–262

Middaugh DP, Hemmer MJ (1984) Spawning of the tidewater silverside, *Menidia peninsulae* (Goode and Bean), in response to tidal and lighting schedules in the laboratory. Estuaries 7:139–148

Nimmo DR, Blackman RR, Wilson, Jr. AJ, Forster J (1971) Toxicity and distribution of Aroclor 1254 in the pink shrimp *Penaeus duorarum.* Mar Biol 11(3):191–197

Perry HM, Van Engel WA (1979) Proceedings of the Blue Crab Colloquium. Gulf States Marine Fisheries Commission. Biloxi, MS

SAS Institute (1987) SAS®/STAT Guide for Personal Computers, Version Six Edition. Cary, NC

Steele P, Perry HM (1990) The blue crab fishery of the Gulf of Mexico, United States: A regional management plan. Number 21. Gulf States Marine Fisheries Commission, Ocean Springs, MS

Stephan CE (1977) Methods for calculating an LC50. In: Mayer FL, Hamelink JL (eds) Aquatic toxicology and hazard evaluation. Amer Soc Test Mat, Philadelphia, PA, pp 65–84

Sulkin SD (1974) Factors influencing blue crab population size: nutrition of larvae and migration of juveniles. Center for Environ Estuar Studies, Chesapeake Biol Lab Annu Rep Ref 74-125, 102 pp

Sulkin SD and Van Heukelem W (1982) Larval recruitment in the crab *Callinectes sapidus* Rathbun: an amendment to the concept of larval retention in estuaries. In: Kennedy VS (ed), Estuarine Comparisons. Academic Press, NY, pp 459–476

Van Den Avyle MJ, Fowler DL (1984) Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic)-blue crab. US Fish Wildl Serv FW/OB-82/11.19. U.S. Army Corps of Engineers, Vicksburg, MS, TR EL-82-4. p 16