FINAL REPORT

SECTION 1 TITLE: Develop Arid West Bioassay Capability for Modification of Water Quality Criteria & Effluent Testing, Award # 01HQGR0113: Project # AZ 961.

SECTION 2 PRINCIPAL INVESTIGATORS: D. J. Baumgartner, K.M. Fitszimmons, S.G. Nelson

SECTION 3 CONGRESSIONAL DISTRICT FOR EACH PI: Seventh, Arizona

SECTION 4 DESCRIPTION INFORMATION

A. Problem and Research Objectives: Most states and Indian Nations establish water quality standards for surface waters within their geographic area of responsibility using a national data base of aquatic organism toxicity data developed by the U. S. Environmental Protection Agency. The aquatic species employed in the EPA bioassays reflect taxa found in perennial streams of the non-arid regions of the nation. A few arid states have been able to justify modified standards by demonstrating that no fish, let alone fish used by EPA (frequently Salmonids), can live in certain arid streams, but other arid states have had to accept EPA numerical criteria for heavy metals, conventional pollutants (e.g., ammonia, chlorine), and toxic organic compounds irrespective of the absence of comparable fish habitat in ephemeral, effluent dependent watercourses of the arid West. Additionally, bioassay species used by EPA to regulate effluent discharges (Whole Effluent Toxicity Tests) are believed not representative of aquatic species found in arid West watercourses.

Conferees at a meeting to discuss research needs for arid West water quality criteria (PCWWM, 1997) concluded bioassay techniques incorporating exposure conditions representative of arid West waters, and use of representative species would improve risk assessment and efficient risk management of river and lake water quality. Without improvements, many municipal and wastewater dischargers may be expending funds to treat effluents to a degree higher than required by the Clean Water Act, or alternatively reusing treated effluents rather than maintaining aquatic and riparian habitats in an ephemeral watercourse. The EPA-funded Arid West Water Quality Research Project (WQRP) has for several years stated that a project of this type may be conducted, but so far the project has not materialized. In addition, the Water Environment Research Foundation (WERF), in collaboration with EPA, has funded a biotic ligand model (BLM) study plan to determine if a more general and fundamental method could more accurately account for metal toxicity than EPA presently uses based on calcium and magnesium concentrations. Unfortunately the BLM study does not include alternate species, nor does it investigate the effects of hardness over 400 mg/l, thus would not address a concern of arid West regulators and dischargers.

B. Methodology: develop and demonstrate maintenance of culture stock of several candidate arid West fish and invertebrate species. Demonstrate survival in mock (control) bioassay procedures, including in relation to standard EPA procedures. Some trial toxicity determinations with a standard toxicant (e.g., copper) will be attempted for two of the species that show promise. These data and demonstrations would then be used to propose longer term research on toxicants and typical arid West waters for grant support by EPA and/or several states.

The Gila chub, *Gila intermedia*, historically found in much of the Gila River watershed, is now restricted to 24 or fewer refugia streams or cienegas in Arizona and Mexico. A captive population of Gila chub is already established at the Environmental Research Lab of the University of Arizona. Approximately 60 fish were collected from Sabino Creek in early 1999 for use in a competitive chub-crayfish feeding trial.We propose to use these fish, which are nearing maturity as the broodstock population.

A population of flannelmouth sucker, *Catostomus latipinnis*, has also being cultured in fiberglass tanks at the Environmental Research Lab. This fish is found in larger, stronger flowing streams of the Colorado River. As with all other native big river fishes, the original range is considerably reduced. Since this population may require a year or longer to reach reproductive maturity, broodstock would be purchased to provide preliminary information in the interim.

Species of the Crustacean Division Eubranchiopoda are among the most characteristic aquatic organisms of temporary bodies of water, such as are common throughout the arid West. The Division consists of three orders: the Anostraca (fairy shrimp), the Notostraca (tadpole shrimp) and the Conchostraca (clam shrimp). Eubranchiopods live in fish-less habitats, and they all produce resting eggs that can withstand prolonged periods of dessication.

Of the three orders we chose to focus on clam shrimp for several reasons. The fairy shrimp, aside from those in the genus *Artemia* (brine shrimp) are difficult to maintain in culture. In contrast, the tadpole shrimp *Triops longicaudatus* can be cultured in the laboratory, and their eggs are commercially available. Techniques for the mass production of *Triops* have been worked out in conjunction with their usefulness in mosquito control, and this techniques could be easily adapted to the culture of this species for bioassays. The clam shrimp, however, fall in between. We have been able to culture them in the laboratory from eggs hatched from playa soils, but little information is available on their mass production; which is needed to develop protocols for routine use in bioassays. We suspect that the mass culture of clam shrimp will be feasible because they have been reported, in some cases, to be a pest in fish ponds.

We also have maintained a culture of *Moina macrocopa* , a species of cladoceran widely used for fish food because of the ease with which they are cultured, their high reproductive rate, and toleration for high density populations. It is a useful species for toxicity tests for these reasons, and because it demonstrates high susceptibility to toxic substances and in particular, metals. Structurally, *M. macrocopa* appear similar to *D. magna* and *D. pulex*. M. macrocopa is also a local species, which we believe will be responsive to metal concentrations and a range of hardness concentrations.

Trial demonstration toxicity tests with copper will be conducted following EPA harmonized guidelines: Ecological Effects Test Guidelines, OPPTS 850.1400, Fish Early-Life Stage Toxicity Test, and Ecological Effects Test Guidelines, OPPTS 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids.

The guidelines for culturing the clam shrimp will be similar to the harmonized guidelines in use bioassays with freshwater daphnids. We propose to conduct a series of replicated laboratory experiments designed to determine optimal conditions for rearing clam shrimp with particular regard to: 1) feed (unicellular algae) concentration; 2) water hardness; and 3) temperature. The experiments will be conducted in 500-ml beakers of water each stocked initially with 10-20 adult clam shrimp.

C. Principal Findings and Significance: Crustacean culture: We have been successful in establishing and maintaining cultures of an ostracode found in samples of soil from Mirror Lake, California. Previously these small crustaceans were identified as clam shrimp (conchostracans), but we recently found that they were actually seed shrimp (ostracoda). Cultures have been established in approximately 15-l containers of distilled water and Tucson municipal water with a 2-cm layer of playa soil. The cultures develop and prosper without supplemental feeding. We used ten replicates at three temperatures each in 150-ml flasks to assess the effect on development of the cultures. Temperatures were 23, 28, and 33 ^{o}C, maintained by water baths equipped with thermoregulators.. By the fourth day ostracode larvae began to emerge. By day ten 48 ostracodes had hatched and developed into adults. Of these, 9 were in the high-temperature group, 17 in the low-temperature group, and 22 were in the middle temperature groups. The counts were significantly different among groups (Chi-square value=22.5; p=0.0041). We found that the ostracode eggs can be easily obtained by isolating adults in glass containers. The orange eggs are negatively buoyant and can be collected from the bottom of the containers. This is a favorable response with respect to the warm watercourse temperatures of the arid West we must represent in the bioassays.

Crustacean bioassays: Early tests were conducted with few replicates (four or eight) to determine generally what concentrations of copper would produce mortality. As we narrowed in on the LC50, more replicates were used (up to 20). Final results indicate that our Ostracode species has a 24-hour copper LC50 of 0.11 mg/l, at a medium water hardness concentration of 75-80 mg/l as $CaCO_3$. Varying water hardness concentrations had little effect on survival, with preliminary tests showing essentially identical survival at hardness between 0 and 300 mg/l.

Fish culture and bioassay: We have successfully maintained cultures of a native species of fish (longfin dace, *Agosia chrysogaster*) and the standard EPA test species (fathead minnow *Pimephales promelas*), and conducted bioassays with a range of copper solutions in moderately hard water. Based on the 96 hour median survival response (LC_{50}) it appears the native species may be more sensitive to copper than the EPA standard. This result, if substantiated with more rigorous bioassays, and a range of hardness concentrations, would be of direct interest to water quality regulatory agencies, as it would tend to justify the approach they have been following for years. It might also placate the wastewater dischargers who have been anticipating, if not asserting, that native species would be less sensitive.

SECTION 5. PUBLICATION INFORMATION : No reports have been published on this work to date. A presentation of the fish bioassay work was made in a student paper at a regional meeting of the American Fisheries Society, with favorable acceptance.

SECTION 6 STUDENT SUPPORT:

Section 104 Base Grant supported

Undergraduate __1________

Masters____1____________

Ph.D.__________________

Post-Doc.______________

Total_____2_____________

SECTION 7 NOTABLE ACHIEVEMENTS AND AWARDS: None