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Utilization of *Odontesthes regia* (Atherinidae) from the South Eastern Pacific as a Test Organism for Bioassays: Study of Its Sensitivity to Six Chemicals

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The assessment of toxicity requires test organisms, particularly fish species, that can account in part for the effects of pollutants on local aquatic fauna. Since fish species are usually part of the superior levels in trophic networks, they have been widely used for ecotoxicological assessments. Among other objectives, this allows for some sort of proxy estimations of the possible effects of toxic substances on human beings.

Toxicity bioassays are used to assess the effects of single chemicals or complex effluents on aquatic life, with the use of indicator species such as freshwater or marine fish (Goodman et al. 1983; Middaugh and Anderson 1993). Salmoniids from freshwater environments, especially the rainbow trout (*Oncorhynchus mykiss*), have been used to study the effects of organic and inorganic substances of complex effluents.

Estuarine species of the family Atherinidae (e.g. *Menidia beryllina*, *M. menidia* and *M. peninsulae*) have been utilized as test organisms to assess the effects of pesticides and fungicides in aquatic environments (Middaugh and Whiting 1995; Hemmer et al. 1992) and the quality of effluents and receiving waters considering acute and chronic toxicity bioassays (U.S. EPA 1988; Klemm et al. 1994).

A species equivalent to *Menidia sp.* for the Southern Hemisphere is *Odontesthes regia*, the common Chilean marine silverside, with a latitudinal distribution along the whole coast of Chile, from Arica (18°20'S) to Punta Arenas (53°08'S). *O. regia* has been utilized by Riveros et al. (1996), to assess the toxicity of waters receiving industrial effluents and by Gaete et al. (1996), to study toxicity levels of heavy metals in Chilean bays with a high degree of anthropic disturbance. This study provides information about the sensitive of *O. regia* to different chemicals, to reinforce its validity as test organism for ecotoxicological assesments.

MATERIALS AND METHODS

Laboratory-reared *O. regia* larvae, hatched from eggs obtained in the Lenga Estuary (36°45'S, 73°08'W, Chile), were used in the bioassays. Egg masses were collected from algal mats of *Gracilaria sp.*, either beach-stranded or from subtidal prairies (using scuba diving).

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Egg masses were kept in a culture chamber at seawater temperature of 13 ± 2°C, with constant aeration and daily water changes. Newly hatched larvae were transferred daily to aquaria with filtered and aerated seawater; this allowed daily control of age and remotion of dead specimens. Larval feeding was initiated at the 6^{th} day after hatching and once the viteline sack was absorbed. This assured optimum conditions of the larvae to be subjected to toxicity tests.

The first two days after viteline sack absorption, larvae were fed twice daily with *Artemia sp.* Instar I stage nauplius, followed on later days with TETRAMIN (sieved through a 0.5 µm mesh) and LANSY R1 (grounded *Artemia*). As the larvae grew it became necessary to increase TETRAMIN particle size.

Daily cleaning of aquaria bottoms was carried out to remove dead specimens and excess food. Water was changed every three days and mild aeration was constantly maintained in the aquaria. All unnecessary manipulation was avoided so as not to stress the larvae.

Larvae for bioassays were chosen among those that exhibited normal behavior (swimming and feeding) and from those aquaria where daily mortality was no greater than 5%. The larvae chosen for each experiment were as homogeneous in age and size (0,6 - 1,2 cm) as possible.

Acute toxicity tests with *O. regia* were 48 hours-long experiments, with larvae exposed to different concentrations of the substance under study. The effect measured was mortality at the end of the exposure time.

Potassium dichromate ($K_2Cr_2O_7$) was used to standardize the toxicity tests. Stock solutions for Copper, Zinc and Cadmium were prepared dissolving the necessary amounts of sulfate salts ($CuSO_4$, $ZnSO_4$ and $CdSO_4$) in distilled water. To get the necessary test concentrations, aliquots from each stock solution were placed in experimental containers and seawater was added to a total volume of 400 mL. Also, stock solutions for the organic compounds Pentachlorophenol (PCP) and 2,4 Dinitrophenol (2,4-D) were prepared and test concentrations were obtained adding aliquots and seawater to a final volume of 400 mL for each container. For PCP bioassays it was necessary to include an acetone control since PCP is soluble in acetone.

Sea water from Coliumo Bay (36°50'S, 72°55'W, Chile), previously filtered, aerated and kept at constant 13°C temperature, was used for dilution and control in all tests. Water salinity was in the range from 28 to 31 ppt and pH was between 7.5 and 7.8. The values of temperature, salinity and pH are the commonly found in the coastal waters were the egg masses were collected. Disposable 500 mL polyethylene containers were used as experimental units in the bioassays with inorganic compounds and 500 mL glass containers were used with the organic compounds. At least five concentrations of one of the experimental solutions were prepared for each experiment, with four replicates per concentration, plus four control replicates with larvae in pure seawater. Ten *O. regia* larvae were added to each test container. Each unit was covered and distributed at random in a water bath thermoregulated at 13° ± 2°C. Photoperiod was maintained at 16:8 h - light: darkness, and individuals were not fed during the experimental time. The number of dead fish per experimental unit was counted at the end of each test. The acceptance criterion was 90% survival in the controls.

All the bioassays were replicated three times o more to obtain the LC_{50} values. Results were analyzed using the PROBIT parametrical statistic (US EPA 1988), that provides LC_{50} or EC_{50} values and their confidence limits.

RESULTS AND DISCUSSION

Because of its wide distribution range along the whole coast of Chile, there are groups with morphological differences whose precise taxonomic status, whether populations or subspecies, is not yet clear. The recent recognition of the species as a valid entity has ended a previous confusion that considered the existence of several species (Dyer et al. 1996; Dyer 1997).

It was not possible to induce spawning of silverside adults in the laboratory, nonetheless, culture of larvae from egg masses collected in the field proved adequate for the objectives of this work. An inconvenience of this method is that it is not possible to have larvae available all year round since *O. regia* is a seasonal spawner. Larval culture was possible from September to April, that is during the spring and summer seasons in the Southern Hemisphere. Females are benthic spawners in shallow coastal waters, sticking egg masses on algal filaments of species such as *Gracilaria sp.* Compared to other fish species, recently hatched larvae are relatively well developed, taking from 5 to 8 days to absorb the viteline sack.

Larval keeping proved to be relatively simple, as they are resistant to handling under laboratory conditions, and it allowed working with larvae of known age. Several diet combinations were tested to keep larvae healthy; the best results in terms of survival and fitness were obtained by using as food *Artemia* nauplius instar I for the first three days after hatching, followed by a combination of LANSY, a commercially available *Artemia*-based food for fish, and TETRAMIN, another food for freshwater fish. According to larval growth the later was sieved to obtain particles of increasing size.

Larvae without viteline sack are recommended for bioassays instead of prelarvae with viteline sack because the latter ones are less sensitive to potassium dichromate (LC_{50} = 136.1 mg/L). Larvae from 7 to 28 days old have been used since no significant difference were found in the response within this age group. Standardization with $K_2Cr_2O_7$ was carried out following the criteria of the US EPA (1988). Table 1 shows LC_{50} – 48 hr values obtained for the six chemicals. Significant differences in sensitivity to the different substances tested can be observed, which can be summarized in the following relationship: **PCP > Cu > Zn > Cd > $K_2Cr_2O_7$ > 2,4-D.**

Table 1: Comparative LC_{50}-48h values (mean ± St. Dev) of organic and inorganic substances, carried out using *Odontesthes regia* larvae as test organisms.

Chemicals	N° of bioassays	LC50 (mg/L)
$K_2Cr_2O_7$	6	88.18 ± 9.99
Zn	4	9.53 ± 1.18
Cu	3	0.057 ± 0.022
Cd	3	18.5 ± 1.4
2,4 – D	2	240.15 ± 47.65
PCP	2	0.023 ± 0.0056

This study suggests that silverside is highly sensitive to toxic substances such as Cu and PCP. Its sensitivity to these substances is on the same level as that determined for *Oncorhynchus mykiss* (Cu = 0.064 mg/L; PCP = 0.068 mg/L), the most widely used freshwater fish species and recommended by the US EPA (1993). Compared to some freshwater fish species studied by the authors (unpublished results), the sensitivity of *O. regia* to the reference toxic substance ($K_2Cr_2O_7$; 88.18 mg/L) is slightly higher than for *Gambusia affinis* (145.42 mg/L), *Cheirodon galusdae* (98.78 mg/L) and *Oncorhynchus mykiss* (172.70 mg/L). On the other hand, it is less sensitive than another atherinid, *Menidia peninsulae* (22 mg/L) (U.S. EPA, 1987).

Our work shows that *O. regia* is more sensitive to copper (57 µg/L) than other atherinids, where the following LC_{50} – 96 hr values have been found: *Atherinops affinis*, 238 µg/L; *Menidia menidia*, 67 – 217 µg/L, and *M. peninsulae*, 140 µg/L (Anderson et al. 1991). However, it is slightly less sensitive to zinc (9.53 against 4.1-8.5 µg/L) and considerably less sensitive to cadmium (18500 against 250-380 µg/L) than *M. peninsulae* (US EPA 1987),

Atherinids have been the most utilized species for bioassays from marine environments, particularly because of the diversity of environments where silverside species can be found and because of their resiliency for laboratory culture and handling. To date there is no native fish species in Chile to comply with international standards for toxicity evaluation. The use of the Chilean common sea silverside (*Odontesthes regia*) is hereby proposed for use in ecotoxicological assessments: its first developmental stages are relatively easy to keep in laboratory; it is widely distributed along the Chilean coast, and it has shown greater sensitivity to the chemicals tested than other species.

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