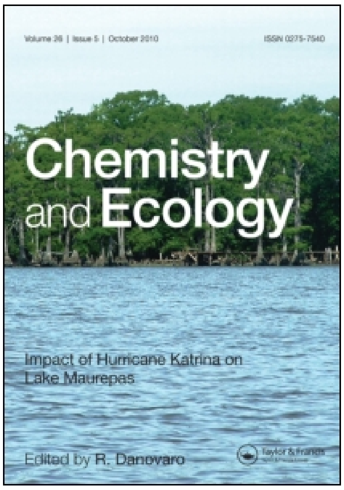

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A proposal of method to evaluate the quality of marine waters: Optimisation of 7 days bioassays using Dicentrarchus labrax (L.) juveniles

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A PROPOSAL OF METHOD TO EVALUATE THE QUALITY OF MARINE WATERS: OPTIMISATION OF 7 DAYS BIOASSAYS USING *DICENTRARCHUS LABRAX* (L.) JUVENILES

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European sea bass juveniles were exposed to cadmium chloride ($CdCl_2 \cdot 2½H_2O$) as reference toxicant. The effects have been evaluated on the survival rates of the fish throughout 7 days. The LC50 (mg/L of cadmium ions) were: 6.17 mg/L (95% confidence interval: 5.44–6.99) after 24 h and 3.43 mg/L (95% confidence interval: 2.87–4.07) after 7 days exposure. This method could be used as a standard procedure to be conducted in short-term tests for estimating the chronic toxicity of marine waters. The high availability of this species at every stage of the productive cycle gives the opportunity to employ *Dicentrarchus labrax* as test organism for defining environmental quality standards.

Keywords: *Dicentrarchus labrax*; Test organism; Bioassays; Ecotoxicology

1 INTRODUCTION

The search for the most suitable aquatic species to carry out toxicity tests has involved the operators of Canada and United States for decades. In these countries, the toxicological analysis of waters and discharges is currently a consolidate tool of control, which is applied concurrently with the chemical analysis (Hamilton, 1976; EPA, 1991).

In Italy, the recent law D.Lgs. n. 152 11/05/1999 introduces the compulsories to carry out tests of toxicity forecasting the use of different bioindicators to evaluate the quality of waters and discharges.

To this purpose, the European sea bass (*Dicentrarchus labrax* L., 1758) was considered to evaluate the sensitivity of this autochthonous marine and brackish water species to toxicants concentrations. For the trials, cadmium chloride ($CdCl_2 \cdot 2½H_2O$) was employed as

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toxicant. This metal is a non-essential element that can have severe toxic effects on aquatic organisms when present in excessive amounts (Alabaster and Lloyd, 1982). In fish, Cd can damage gills, resulting in skeletal deformities and disturb calcium balance (Hutchinson *et al.*, 1994; Hollis *et al.*, 1999; Romeo *et al.*, 2000). Cadmium chloride has been recommended by the Environmental Protection Agency as reference toxicant in tests to evaluate the long-term toxicity in effluents and outlet waters (EPA, 1993; Klemm *et al.*, 1994). The toxicity of cadmium chloride under different environmental conditions has been estimated in a few organisms such as water flea (*Daphnia magna* and *Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*) (Call *et al.*, 1998) and rainbow trout (*Salmo gairdneri*) (Calamari *et al.*, 1980) whereas no study on European sea bass has been reported yet.

This work reports the results obtained by sea bass juveniles used to optimize a toxicity test procedure, carried out under laboratory conditions, with the aim of determining lethal concentrations at 7 days of cadmium chloride exposure.

2 MATERIALS AND METHODS

2.1 Fish and Rearing Conditions

To ensure availability of animals for the tests, juvenile sea bass were held under optimal laboratory conditions: the fish were maintained for 7 days in quarantine, in order to detect possible diseases and mortality caused by transportation stress and/or the acclimation to the new environmental conditions. After the quarantine, 200 fish were housed in 100 L recirculating aquaria, with a complete water exchange occurring every 3 h. The aquaria were provided with a mechanical and biological filtration system and a device for water temperature regulation. Photoperiod was fixed at 16 h light and 8 h dark, and average water temperature was maintained at $20 \pm 0.5\,^{\circ}C$. The activation of the biological filters required an accurate preliminary preparation and the development of the bacteria populations on the filtering substrate required 40 days.

After the acclimation period (3 days), fish were fed using *Artemia* nauplii and high protein content (54% dry matter) extruded diet. In all tanks, the stocking density was maintained at 0.24 g/L. The main water physico-chemical parameters were monitored daily in all the aquaria (pH = 7.8–8.5; salinity = 30 ± 2 ppt; dissolved oxygen = 6–8 mg/L; $N\text{-}NH_3 < 0.01$ mg/L; $N\text{-}NO_2 < 0.01$ mg/L; $N\text{-}NO_3 < 80$ mg/L).

2.2 7 Days Toxicity Tests Procedure

The tests were carried out using sea bass juveniles, weighing 0.12 ± 0.02 g and sizing 2.43 ± 0.05 cm, regularly fed with newly hatched *A.* nauplii until the day before the start of trials included. The solutions were prepared using synthetic seawater, at 30‰ ± 1‰ salinity, by diluting Instant Ocean® salt in deionised water. The procedure was applied following standard methods (Klemm *et al.*, 1994; Roncarati *et al.*, 2001a) (see Tab. I).

Preliminary tests were carried out to determine the final range of cadmium chloride concentrations for the definitive experiment. These concentrations (25.1, 12.6, 6.31, 3.16, 1.58 mg/L of cadmium ions) and the values of cadmium were also analysed in the water following APHA (1995) methods and a small difference was showed between the concentration added and that instrumentally measured in the solution (−0.005 mg/L).

Test chambers used for sea bass contained 1000 mL of solution. Ten fish were transferred randomly to each test chamber, with caution to avoid damages to the animals. The stocking density in the test chambers was 1.2 g/L.

TABLE I Conditions adopted in 7 days toxicity tests for European sea bass.

Biological stage of the organism tested	Juveniles (0.12 ± 0.02 g; 2.43 ± 0.05 cm)
Type of bioassay	Static renewal
Reference toxicant	Cadmium chloride
Temperature	20 ± 1 °C
Light intensity	500–800 lux
Photoperiod (L:D)	16 h L: 8 h D
Volume solution bioassay	1000 mL
n. organisms/test and control	10
n. replicas/concentration	3
Type of feeding during the test	*Artemia* nauplii
Aeration	Absent
Dilution water	Artificial seawater
Renewal of test solutions	Daily
Test duration	7 days
Response	Mortality
Test acceptability criteria	80% or greater survival in controls

The tests were performed in triplicate with one control for each working concentration. The solutions were renewed daily using freshly prepared solutions. During the assay, the test juveniles were fed with *Artemia* nauplii once a day from day 0 to day 6. The dissolved oxygen concentration was measured at the beginning and end of each 24-h exposure period. Daily and at the end of the tests, the dead organisms were counted. In control groups, no cadmium chloride was added and water quality was kept the same as the experimental treatments.

Throughout the 7 days, every 24 h, LC50 were calculated both by graphic representation and statistical method, using ToxStat software package, based on Trimmed–Spearman–Karber (TSK) test and Probit analysis.

3 RESULTS AND DISCUSSION

The LC50 (mg/L $CdCl_2 \cdot 2½ H_2O$) were: 6.17 mg/L (95% confidence interval: 5.44–6.99) after 24 h; 3.94 mg/L (95% confidence interval: 3.30–4.68) after 96 h and 3.43 mg/L (95% confidence interval: 2.87–4.07) after 7 days exposure (Fig. 1). The survival rate in the control solutions was 100%.

These data are interesting when compared to those obtained exposing other marine fish species to short-term tests to cadmium chloride (CTN AIM, 2000). Indeed, in 96 h short-term tests the species tested (*Liza vaigiensis*, *Mugil cephalus*, *Platichthys flesus*) showed a

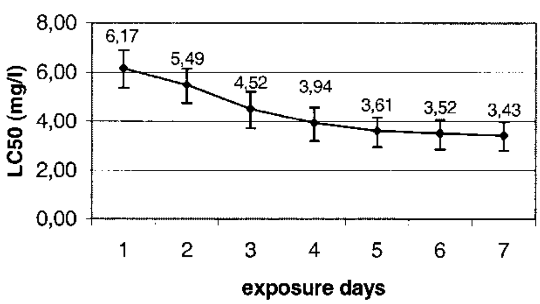

FIGURE 1 LC50 and confidence limits in European sea bass juveniles exposed to cadmium chloride (mg/L cadmium ion).

higher tolerance (5.2, 6.6, 24.4 mg/L, respectively) (Larsson *et al.*, 1981; Denton and Burdon-Jones, 1986; Koyama *et al.*, 1992) to this compound than the fish tested in the present investigation.

This work allowed to evaluate the ecotoxicological assays to be carried out exposing juveniles sea bass to cadmium chloride as reference toxicant.

The European sea bass could nicely withstand the conditions of the aquaria for short-term tests in agreement with Person Le-Ruyet *et al.* (1995) that estimated the acute ammonia toxicity in acute bioassays; however, these authors used juveniles significantly higher (from 6 to 163 g) than those employed in our tests (m.b.w. = 0.12 g).

The procedure applied in this study on European sea bass showed the suitability of this autochthonous species to perform ecotoxicological tests, as required by D. Lgs. 152/99. This method could be used as a standard procedure to be conducted in short-term tests for estimating the chronic toxicity of marine waters, brackish waters and sediments.

In the last ten years, the rearing of the sea bass (*D. labrax* L.) has experienced an exponential growth in Europe where the production increased from 1200 t in 1990 to 35,000 t in 2001. Most of them are commercialized in Italy, which is the largest consumer country of sea bass in the European Union (Roncarati *et al.*, 2001b). The high availability of this species at every stage of the productive cycle gives the opportunity to employ *D. labrax* as test organism for defining environmental quality standards.

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