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Detection of Range Finding Test of Mercury Chloride in Yellowfin Sea Bream (*Acanthopagrus latus*)

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Abstract: Toxicity tests allow the determination of pollution effects, providing direct evidence of the biological responses of marine organisms to contaminants. Fifty four yellow fine sea bream (Acanthopagrus latus) all immature male in same size (120 g final body weight average) were obtained from Mahshahr creeks with hooks in a Upon capture. In laboratory Fish maintained in a seawater re-circulatory system (300-L tanks) equipped with physical/biological filters and with aeration. All samples were acclimatized for one weeks in a 15 aerated fiberglass tank containing 46 ppt saltwater maintained at 25 C under a constant 12:12 L:D photoperiod. Acclimatized Fish were fed daily with a live feed (fresh shrimp) and daily we checked water quality and water parameters. $HgCl_2$ tested concentrations were 20, 50, 100, 200, 500, 1000, 2000, 5000 and10000 µg/l, Groups of six male yellow fine sea bream (120 g) were exposed for 96 h to each of the Range finding test for LC50, in fiberglass tank equipped with aeration with 100 l of test medium. The control group was exposed to filtered sea water in similar conditions. Test medium was not renewed during the assay and no food was provided to the animals. Values of pH, Temperature and salinity were measured at time 0, 24, 48, 72 and 96 h. At the end of the bioassay, Range values were determined as 500-1000 µg/l (fifty percent of mortality between 500 and 1000). Range finding test values indicated that mercury is more toxic to A. latus than other same marine spices. Range obtained in the present study compare with corresponding values that have been published in the literature for other species of fish, show different Range of mercury in different species and even different time, but what is important, lower value of range finding test for *A. latus* compare with most species and confirm sensitively of *A. latus* to low mercury doses.

Key words: Range finding test · Mercury Chloride · *Acanthopagrus latus*

INTRODUCTION

Aquatic ecosystems are typically monitored for pollution of heavy metals using biological assays. Fish species are often the primary consumers in any aquatic ecosystems and thus metal concentration in fish can act as an environmental indicator of the state of any aquatic system. Aquatic organisms have been reported to accumulate heavy metals in their tissues several times above ambient levels. Fishes have been used for many years to determine the pollution status of water and are thus regarded as excellent biological markers of metals in aquatic ecosystems.

Mercury (Hg) is a liquid metal at ambient temperatures and pressures. It forms salts in two ionic states mercury (I) and mercury (II). Mercury (II), or mercuric salts, are much more common in the environment than mercury (I) or mercurous salts. These salts, if soluble in water, are bioavailable and considered toxic. Mercury also forms organometallic compounds, many of whichhave industrial and agricultural uses [1].

Mercury in fish was already recognized as a public health and ecological problem in the 1960's. It was commonly assumed that local point sources (industrial effluent, utility emissions, fungicide

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applications) were the main sources and many studies focused on waters with nearby point source contamination.

Although mercury chloride is not the most toxic mercury compound in the marine environment [2], it is the key form between the gaseous metal form transported through atmosphere and the methylmercury form that bioaccumulates in organism. Once it enters into the organism, mercury can draw various immunotoxic effects [2].

Toxicity tests allow the determination of these effects, providing direct evidence of the biological responses of marine organisms to contaminants. Due to the fact that organisms from different species vary in their sensitivity towards chemical substances, it is difficult to set standards for protection of species with regard to pollutants in the environment. Extrapolation from one species to another is, therefore, difficult if their relative sensitivities are not known [3].

The present study was conducted to determine the acute toxicity of the heavy metal compound $HgCl_2$ in a statistic system to the marine fish *Acanthopagrus latus*. This species was selected for bioassays because it can easily be raised under laboratory conditions. It fulfills most of the requirements of a model species and is available throughout the year.

MATERIALS AND METHODS

Fifty four yellow fine sea bream all immature male in same size (120 g final body weight average) were obtained from Mahshahr creeks with hooks in a Upon capture, (only healthy fish, as indicated by their activity and external appearance, were used in the experiments) the fish were maintained alive on board in a fiberglass tank and on return to shore transferred to a 300-L aerated vat filled with sea water for transport back to the nearby laboratory. In laboratory Fish maintained in a seawater re-circulatory system (300-L tanks) equipped with physical/biological filters and with aeration to the Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran from October to November.

All samples were acclimatized for one weeks in a 15 aerated fiberglass tank containing 46 ppt saltwater maintained at 25 C under a constant 12L:12D photoperiod. Acclimatized Fish were fed daily with a live feed (fresh shrimp) and daily we checked water quality and water parameters.

$HgCl_2$ tested concentrations were 20, 50, 100, 200, 500, 1000, 2000, 5000 and10000 µg/l, Groups of six male yellow fine sea bream (120 g) were exposed for 96 h to each of the range finding test for LC50, in fiberglass tank equipped with aeration with 100 l of test medium. The control group was exposed to filtered sea water in similar conditions.

The bioassay was performed in a temperature (25±1 _C) and under a natural photoperiod (12hL: 12hD) controlled room. Test medium was not renewed during the assay and no food was provided to the animals. Values of pH, Temperature and salinity were measured at time 0, 24, 48, 72 and 96 h.

RESULTS

There was 100% mortality at 10000 µg/l concentration within the first 4h after dosing and 100% mortality at 5000 µg/l within the 14h whereas 100% mortality for 2000 µg/l was 42h and for 1000 µg/l was 54h.

The mortality of yellowfin sea bream for mercury chloride doses 20, 50, 100, 200, 500, 1000, 2000, 5000 and 10000 µg/l were examined during the exposure times at 24, 48, 72 and 96 h for range finding test (Table 1). Fish exposed during the period 24-96h had significantly increased number of dead yellowfin sea bream with increasing concentration. There were considerable differences in number of dead fish between the duration 24-96 in each. because of no mortality at 500 µg/l and 100% mortality at 1000 µg/l, we finding that main range is between 500-1000 µg/l, the mortality of yellowfin sea bream for mercury chloride were examined during the exposure times at 24, 48, 72 and 96 h for range finding test are in Figure 1-4.

Table 1: Cumulative mortality of yellowfin sea bream (n=6, each concentration) at Range finding test

	No. of dead yellowfin sea bream			
Concentration (µg/l)	24h	48h	72h	96h
Control	-	-	-	-
20	-	-	-	-
50	-	-	-	-
100	-	-	-	-
200	-	-	-	-
500	-	-	-	-
1000	1	3	6	6
2000	2	6	6	6
5000	6	6	6	6
10000	6	6	6	6

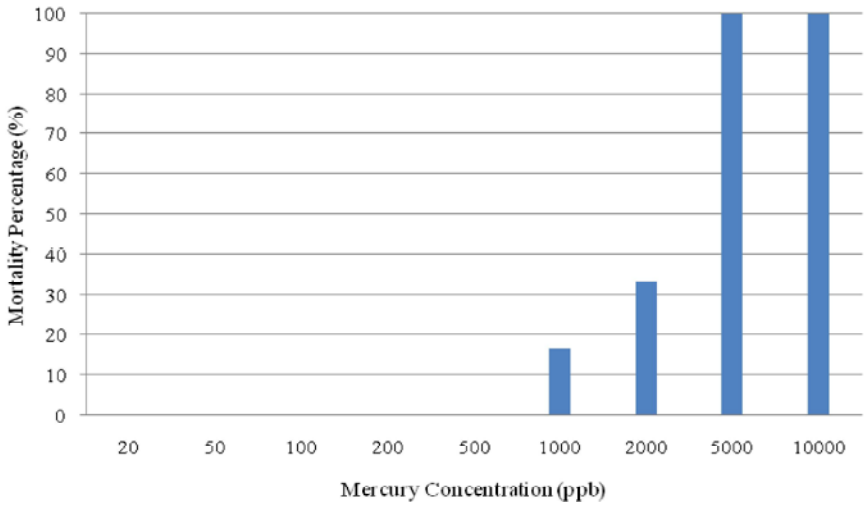

Fig. 1: 24h Mortality percentage of yellowfin sea bream exposed to mercury chloride

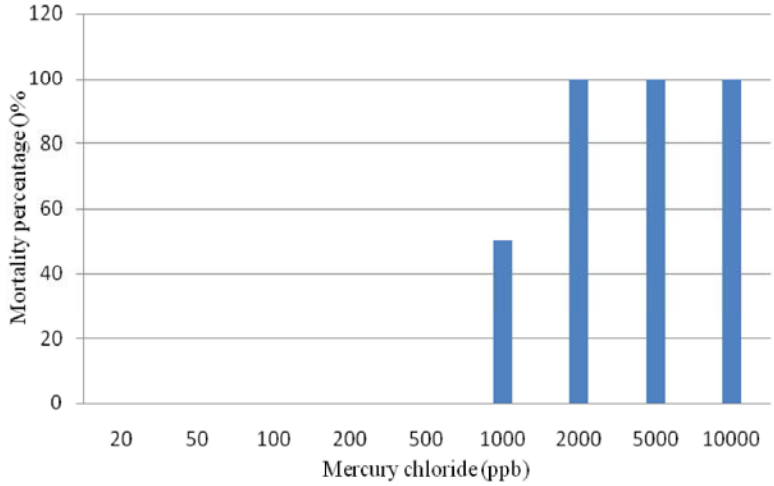

Fig. 2: 48h Mortality percentage of yellowfin sea bream exposed to mercury chloride

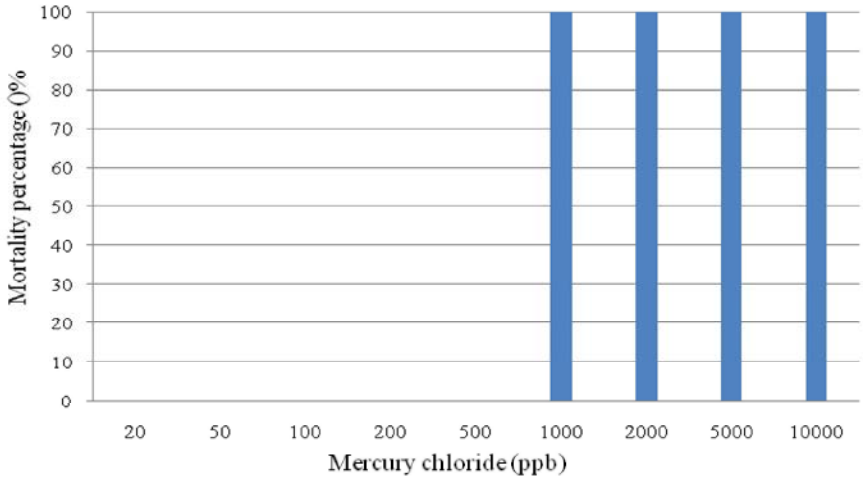

Fig. 3: 72h Mortality percentage of yellowfin sea bream exposed to mercury chloride

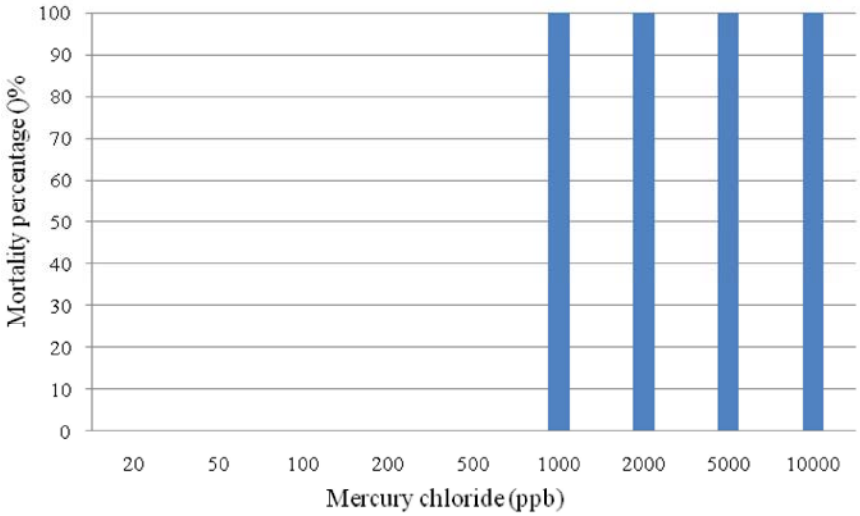

Fig. 4: 96h Mortality percentage of yellowfin sea bream exposed to mercury chloride

DISCUSSION

Toxic effects of mercury and its compounds depend on the chemical form of mercury. Organic forms of mercury are generally more toxic to aquatic organisms than are inorganic forms [1]. $HgCl_2$ can be converted into highly toxic methyl mercury by methylation through chemical or biological processes [1].

Factors influencing mercury levels can be divided into exogenous (characteristics of the water body) and endogenous (characteristic of the individuals or species). Exogenous factors include pH, sulfur and organic matter (e.g., dissolved organic carbon). Endogenous factors include species, habitat and food preferences, metabolic rate, age, growth rate, size, mass and diet.

According to the Gooley *et al*. [4], mercury is one of the concern metals in aquaculture and has 10-40 µg/l of LC_{50} with only 1µg/l for safe levels, whereas LC_{50} value for other heavy metals is higher than mercury (cadmium 80-420, cooper 20-100, zinc1000-10000, lead 1000-40000 µg/l). Chowdhury *et al* [5] show the 96-h LC_{50} for the juvenile trout as11 µg/l (95% CI = 9.2 - 11.9 µg/l).

The 96-h LC_{50} value for catfish exposed to Hg2+ under static test was determined to be 570 µg/l [6]. The 96-h LC50 value of mercury chloride for Chub mackerel (*Scomber japonicus)* was found as 205 µg/l and 96-h LC_{50} for trout 814 µg/l [7]. On the estuarine fish *Pomatoschistus microps,* LC_{50} of copper and mercury at 96 h were 568 µg/l and 62 µg/l, respectively [8].

The concentrations of trace metals that resulted in mortality of *H.rubra* were investigated by exposing juveniles to acute concentrations of Cu, Zn, Hg and Cd for 96hr. Hg resulted in more sudden mortality rate after 24hr exposure compared to Cu yet produced a 96-h LC_{50} of 173µg Hg/L [9].

EPA studies on many aquatic species show vast range of LC50 for mercury chloride, which for saltwater fish was 36 µg/l (juvenile spot) to 1678 µg/l (flounder), that was higher than saltwater invertebrate 3.5 µg/l (mysid shrimp) to 400 µg/l (soft clam). This result emphases that yellowfin sea bream is sensitive to mercury chloride and have low Range value.

According to FAO/UNEP [10], the 96-h LC_{50} values of mercury chloride are for cat fish 350 µg/l, rainbow trout 220 µg/l, striped bass 90 µg/l and brook trout 75 µg/l. the 96-h LC_{50} values of mercury chloride are 37 µg/l for fathead minnow, 160 µg/l for bluegill sunfish, 903 µg/l for rainbow trout, 200 µg/l for rainbow trout and lower in invertebrate, 2 µg/l for crayfish, 5 µg/l for cladocera, 10 µg/l for Gammarus, 5 µg/l for blue mussel, 15 µg/l for prawn and 3 µg/l for limpet [11].

Rathore and Khangarot [12] reported that the acute toxicity of $HgCl_2$ increases with increase in temperature. Cairns *et al*. [13] reported similar trends for other metals. Khangarot and Ray [14] also observed that the toxicity of copper abruptly decreased with an increase in pH of the Cu-containing medium. Acute toxicity studies are the very first step in determining the water quality requirements of fish. These studies obviously reveal the toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. Therefore, studies demonstrating the sensitivity of genotoxic effects of heavy metals in aquatic organisms, particularly in fish are needed. Thus, it can be concluded from the present study that fish are highly sensitive to $HgCl_2$ and their mortality rate is dose dependent.

Comparison of values reported earlier with those obtained in the present study may not be meaningful because various factors may influence bioassay techniques like differences in fish(e.g., species, weight, size) and other environmental factors (temperature, variations in pH of the water, total hardness of water, dissolved oxygen). Sprague [15] observed variability in acute toxicity even in a single species and single toxicant depending on the size, age and condition of the test species along with experimental factors. Gupta *et al*. [16] reported that the differences in acute toxicity may be due to changes in water quality and test species.

Chronic toxicity values are much lower than acute values and highlight the adverse effects of relatively low concentrations of mercury in water (i.e., < 1 µg/L) [9].

In aquatic toxicology, if LC50 concentration is smaller than 1000 µg/l, the chemical is highly toxic and if between 1000-10000 µg/l, then it is considered to be moderately toxic [17], therefore we report mercury chloride to be highly toxic to yellowfin sea bream and may cause many damage in this Fish.

Range values indicated that mercury is more toxic to *A. latus* than other same marine fishes. Range obtained in the present study (500-1000 µg/l) compare with corresponding values that have been published in the literature for other species of fish, show different Range of mercury in different species and even different time, but what is important, lower value of Range for *A. latus* compare with most species and confirm sensitivity of *A. latus* to low mercury doses.

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