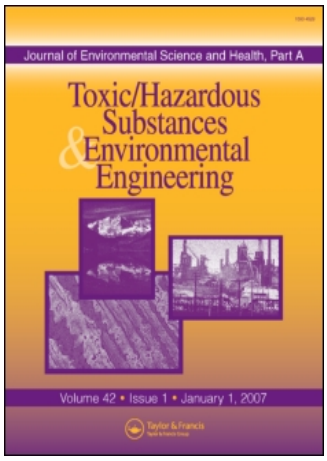

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Evaluation of acute copper toxicity during early life stages of gilthead seabream, *Sparus aurata*

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In this study, the effects of exposure to copper (mortality and morphological alterations) on the early life stages of the gilthead seabream, *Sparus aurata*, were examined. Eggs/embryos and larvae were exposed to nominal concentrations of copper ranging from 0.0001 to 10 mg/L Cu (II) in the tests with eggs/embryos and 0.025 to 0.5 mg/L Cu (II) in the test with larvae. Duration of the assays was 48 hours for embryos and 96 hours for larvae. A high percentage of mortality was observed in embryos exposed to 0.1 mg/L (97.2%) and in larvae exposed to 0.5 mg/L (100%). The embryos proved the most sensitive to copper for the same duration of exposure. The acute toxicity expressed as LC_{50} 48 hours was 0.054 (0.048–0.058) mg/L for embryos and 0.261 (0.182– 0.375) mg/L for larvae. Morphological alterations or abnormalities in embryos included irregular shapes of chorion, opacity and vitellus retraction/degeneration. In larvae we observed poor capacity to swim, trembling, myoskeletal defects, opacity and exophthalmia. Histopathological alterations are observed in *S. aurata* larvae. Mucous cells of the digestive tissue present a severe alteration with an increment of exudates. A great cellular disorganization in the renal tissue is observed. Results from this work indicate the high sensitivity of early life stages of *Sparus aurata* to copper (II) and the persistence of sublethal effects.

Keywords: Toxicity; Copper (II); Embryos; Larvae; *Sparus aurata*; LC_{50}; Mortality; Sublethal Effects.

Introduction

The ocean is the final destination of many discharges of anthropogenic origin, often resulting in toxic concentrations of contaminant in coastal waters. The nature of toxic substances is diverse: surfactants, pesticides, fertilizers, hydrocarbons, radioactive compounds, heavy metals, etc. Metals enter surface waters from a variety of sources including industry discharges, domestic sewage, non-point runoff, urban storm runoff and atmospheric precipitations. Metals encompass a wide range of non-degradable and highly persistent substances and also those bioaccumulate in the organisms.

Copper, which occurs in natural waters primarily as the divalent cupric ion in free and complexes forms, is a minor nutrient for both plants and animals at low concentrations but is toxic to aquatic life at concentrations only slightly higher.[1] In unpolluted surface waters, copper is present at medium concentrations of around 5 μg/L,[2] but in coastal areas where discharges are common, the concentration of this metal is highly variable and can easily reach toxic levels for many aquatic organisms.[3]

Copper sulfate ($CuSO_4$) is one of the most widely used algaecides for phytoplankton control in fish ponds, reservoirs and lakes, as well as an herbicide.[4,5] At present, the extensive use of copper has raised concern about the consequences of copper treatment or natural or farmed organisms.[6–8] The toxicity of copper on adult fish, its accumulation within tissues, and its impacts on physiological mechanisms have been well studied,[9–14] including gilthead seabream.[15,16] Copper toxicity disrupts ion homeostasis and growth in juvenile and adult fish.[17–24] There is limited information relative to the effects of copper on aquatic organisms exposed during the earliest life stages.

The teleost *Sparus aurata* is one of the most abundant and representative species of the Atlantic and Mediterranean coasts.[25–27] In the last years, the culture of the teleost *Sparus aurata* has reached great economic and commercial interest.[28] Due to its wide distribution, importance from the commercial point of view, disponibility and

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the wide knowledge about this specie, in the last years Sparus aurata is being used to test the toxicity of chemicals used in aquaculture or pollutants that reach the marine environment.[29–33] In this study, we assess the acute toxicity of copper on gilthead seabream, embryos and larvae, and the associated morphological alterations.

Materials and methods

Experimental animals

Fertilized eggs (1–2 h post-fertilization (HPF)) and larvae (6–10 h post-hatching (HPH)) of gilthead seabream, *Sparus aurata,* were obtained at the Laboratory of Marine Culture at the Marine and Environmental Sciences Faculty of the University of Cadiz. Embryos (size: 0.8 mm) and larvae (size: 1 mm) selected for tests were checked under a stereomicroscope to avoid the use of organisms with obvious disease or apparent stress (due to handling or culture conditions) that could fake the results of the test.

Toxicity testing

The experimental design was adapted to the procedure proposed by USEPA[34] to obtain normalized results for life-cycle studies in fish embryos. Static (i.e., no water replacement) acute toxicity tests using different copper concentrations, were performed. Exposure times were 48 hours for embryos test (larvae hatching after 48 h) and 96 hours for larva test (larvae open the mouth to start the exogenous feeding when the endogenous yolk sac reserves are depleted).

The test copper solutions ($CuSO_4 \cdot 5H_2O$ PRS Pure. Panreac, S.A.) and the controls of the tests were prepared with filtered natural and unpolluted seawater (45 μm fiberglass filter) from Sancti Petri Beach (South Atlantic Spanish Coast) (copper (II) concentration lower than 0.1 μg/L). Concentrations of the test are expressed in mg/L Cu (II).

Then, 1 L of each test solution (and controls) was added in 2 L glass vessels at nominal concentrations of 0.0001, 0.001, 0.01, 0.025, 0.050, 0.075, 0.1, 1 and 10 mg/L for embryos, and 0.025, 0.050, 0.075, 0.1, 0.25 and 0.5 mg/L for larvae. Three replicates for each concentration including control group were realized.

Fifty embryos and fifty larvae were placed in each vessel with continuous slight aeration. Temperature was maintained at 19 ± 1°C, salinity of 39.6 ± 0.1, pH 8.2 ± 0.3, photoperiod 12 light/12 hour darkness, and dissolved oxygen 95% saturation. These physical and chemical parameters were monitored daily in each vessel. Dead and living embryos were counted after 24-hour and 48-hour exposure. In the case of larvae test, dead and living animals were recorded daily throughout the 96-hour period. Dead embryos and larvae (deposited in the bottom of the vessels) were removed daily from the test vessels. Opaque and submerged eggs were characterized as dead embryos. Larvae were considered dead when immobility, opacity and no heartbeat were observed. Dead eggs and embryos were removed immediately to avoid fungal infection of the other eggs. The survival, mortality and corrected mortality percent were calculated for each concentration:

$$\%\ \text{Survival} = \frac{\text{N°living embryos/larvae}}{\text{N°total of embryos/larvae exposed}} \cdot 100$$

$$\%\ \text{Mortality} = 100 - \%\text{Survival}$$

$$\%\ \text{Corrected Mortality} = \frac{\%\ \text{Mortality} - \%\ \text{Control Mortality}}{100 - \%\ \text{Control Mortality}} \cdot 100$$

Morphological alterations

A microscopy (Leica Leitz DMRBE) with a digital camera coupled (SONY DKA-AC30) was the method employed to observe the morphological alterations in *Sparus aurata* embryos and larvae exposed to copper at different concentrations and exposure times.

Histopathology

Moribund larvae from 0.025 and 0.075 mg/L copper test solutions were studied for histopathological manifestations in different tissues. Samples were fixed in 10% formalin and then processed, sectioned, and stained using standard protocol.[47]

Statistical analysis

Percentages of corrected mortality data were used in the statistical analysis. The computer model (Probit Program Version 1.5) prepared by the US Environmental Protection Agency was used for the calculation of different LC_p (p = percentage of mortality). This program calculates the mean χ^2 statistic for heterogeneity of results. If the tabulated value is significantly greater than the calculated value, the results of the experiments fit the model and the results are statistically valid. The program also estimates the mean linear regression parameters and uses them to calculate the mean LC_p and associated 95% confidence intervals.[34]

Results and discussion

Lethal Toxicity of the copper solutions

In both, embryos and larvae tests, control mortality were less than 10%. This percentage was selected previously as quality control of the toxicity tests. Percent of corrected mortality with controls mortality for embryos and larvae (Table 1 and 3) are plotted versus copper concentration to get the response curve (Figs. 1 and 4). The LC_p values

Table 1. Percent of corrected mortality (average and standard deviation) versus copper concentrations in the embryos test

Concentration (mg/L)	*% Corrected mortality (Mean ± S.D. n = 3)*	
	24-h	*48-h*
0 (Control) (*)	1.66 ± 0.57	2.6 ± 1.15
0.0001	1.36 ± 1.17	9.11 ± 2.24
0.001	2.73 ± 1.16	17.56 ± 2.91
0.01	6.14 ± 2.00	31.64 ± 3.59
0.025	33.19 ± 5.30	49.90 ± 18.56
0.050	35.07 ± 6.11	50.17 ± 11.33
0.075	47.80 ± 17.97	85.05 ± 13.45
0.1	97.20 ± 4.84	99.27 ± 1.25
1d	100 ± 0	100 ± 0
10	100 ± 0	100 ± 0

(*) In the case of the controls, data correspond to the percent of mortality non corrected.

Table 2. Estimated LC values and 95% confidence limits for embryos 24 and 48 hours exposed to copper

Toxicity expression	*Concentration mg/L Cu (II) (lower 95%-upper 95%)*	
	24 hours	*48 hours*
LC_1	0.039 (0.017–0.049)	0.028 (0.019–0.034)
LC_5	0.047 (0.024–0.056)	0.034 (0.025–0.040)
LC_{10}	0.052 (0.031–0.060)	0.037 (0.029–0.043)
LC_{15}	0.056 (0.038–0.063)	0.040 (0.032–0.046)
LC_{50}	0.076 (0.068–0.093)	0.054 (0.048–0.058)
LC_{85}	0.103 (0.087–0.195)	0.072 (0.066–0.081)
LC_{90}	0.110 (0.091–0.234)	0.077 (0.070–0.089)
LC_{95}	0.123 (0.098–0.308)	0.085 (0.076–0.102)
LC_{99}	0.149 (0.111–0.517)	0.103 (0.089–0.134)
χ^2 for heterogeneity	1.929	1.112

Tabular $\chi^2 = 3.841$ at 0.05 confidence level.

(LC_1–LC_{99}) and 95% confidence intervals for different exposure time obtained in the Probit analysis are given in Table 2 (embryos) and Table 4 (larvae). The LC_{50} values showed gradual decrease with increase in time. In general, the increase in percent mortality was related to both time and copper concentrations. The highest mortality occurred at 48 hour (embryos) and 96 hours (larvae) in highest concentration of the metal.

Embryo stage was more sensible than larva. For an specific exposure time, e.g., 48 hours, the LC_{50} values of copper for embryos and larvae were found to be 0.054 (0.048–0.058) and 0.261 (0.182–0.375) mg/L Cu (II). So, LC_{50} for embryos was 4–5 times less than LC_{50} for larvae. For a specific concentration, e.g., 0.1 mg/L, the mortality percentage for embryos and larvae at 48 hours was 99% and 25% respectively. The greater mortality on embryos in this experiment can due to the chorion of fish embryos is considered to be easily permeable to inorganic ions and water, contrary to the perivitelline membrane.[35]

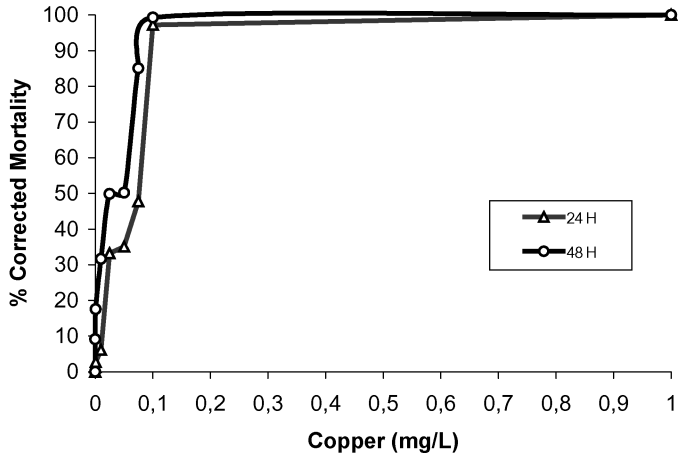

Fig. 1. Relationship between copper concentration and percentage of corrected mortality in *Sparus aurata* eggs.

The property of the chorion makes possible the maintenance of a hypo-osmotic environment around the embryo. An active regulation appears after gastrulation and is fully developed by yolk plug closure and contributes then also to keeping the egg hypo-osmotic. The deleterious effects of copper on chorion could affect the development of the embryo. This hypothesis is supported by the white coloration observed in the chorion, indicating denaturalization of the proteins of which it is composed.[36] The sensitivity of *S. aurata* was found to fall within the range of other fish species reported in literature. For example, we can find data like that reported by Flik et al.,[37] who found in tests with carp larvae (*Cyprinus carpio*) a mortality lower than 50% after an exposition of 48 hours to 0.05 mg/L; Hamilton and Buhl[38] conducted tests with flannelmouth sucker larvae (*Catostomus latipinnis*) and they obtained a 48-hour LC_{50} of 0.175 mg/L; Krishnani et al.[39] observed in *Lates calcarifer* of 11 ± 3 mm, a 96-hour LC_{50} of 1,3 mg/L; and Nguyen and Janssen[40] observed that none African catfish (*Clarias gariepinus*) larvae reached 96 hours LC_{50} of 2.5 mg/L.

Morphological alterations

No statistical analysis was executed to determine NOEC or LOEC concentration by using observed abnormalities or morphological alterations as biological response to the toxicant. However, it is possible to approach these concentrations from a qualitative or descriptive point of view. Microphotography showed abnormalities in both embryos and larvae. Morphological alterations in embryos include: irregular shapes of chorion, opacity, vitellus retraction and degeneration, and decreased buoyancy. In larvae, morphological alterations comprise: poor capacity to swim, trembling, myoskeletal defects, opacity and exophthalmia.

Table 3. Percent of corrected mortality (median and standard deviation) versus copper concentrations in the larvae test

	% Corrected mortality (Mean ± S.D. n = 3)			
Concentration(mg/L)	*24-h*	*48-h*	*72-h*	*96-h*
0 (Control) (*)	4.33 ± 1.52	4.66 ± 1.15	4.66 ± 1.15	4.66 ± 1.15
0.025	0.70 ± 1.22	9.58 ± 2.66	19.85 ± 2.23	26.48 ± 2.28
0.050	1.46 ± 1.26	13.96 ± 1.12	23.51 ± 5.19	37.45 ± 2.90
0.075	2.29 ± 2.38	16.26 ± 7.08	42.71 ± 4.46	48.58 ± 3.37
0.1	3.00 ± 2.61	24.99 ± 1.08	59.55 ± 3.28	63.96 ± 3.27
0.25	11.61± 2.97	37.48 ± 1.62	72.92 ± 12.45	88.40 ± 11.97
0.5	20.31± 5.79	78.71 ± 2.89	100 ± 0	100 ± 0

(*) In the case of the controls, data correspond to percent of mortality non the corrected mortality percent.

Figure 3 shows the retardation in the development of embryos. This retardation is proportional to the increase of the concentration of copper and exist a clear copper concentration-injury grade relationship. From the concentration of 0.075 mg/L, the larvae were no hatched. In the control vessels, embryos, as well as their chorions, appeared transparent (Fig. 2A). When the copper concentration reached lethal limits, the dead embryos turned from transparent to white, indicating denaturalization of the proteins of which they are composed.[36] Finally, at the highest test concentrations, even the chorions turned white, the embryo appeared as a cellular mass, and the structures previously developed have disappeared (Figs. 2B and 2C).

The effects of copper on eggs buoyancy (expressed as vertical movement) have been observed in eggs of cod, *Gadus morhua.*[41] It is known that the pelagic embryos of fish have an ability to actively regulate their buoyancy. The gain or loss of water is one important factor and one way it may be regulated is by change of osmolarity in the ripening egg by control of protein hydrolysis.[42]

In this study we observed that the regulation of egg buoyancy is disturbed by exposure to the copper and this is a factor very important for the survival of the egg. This may be due to an increased permeability of the perivitelline membrane and impairment of active ion excretion leading to loss of water and inflow of ions into the egg to make the perivitelline liquid less hypo-osmotic and the egg less buoyant.[41]

In the case of larvae (Fig. 5), the morphological alterations begin to be observed at 0.05 mg/L (reduction of pigmentation) and 0.1 mg/L copper (spinal cord deformation).

Flik et al.[37] in their studies on carp larvae, *Cyprinus carpio,* exposed to 0.0008 mg/L Cu exhibited a 10% incidence of spinal cord deformation at 24 HPF, and this increased to 34% and 37% at 48 and 72 HPF, respectively. Larvae exposed to 0.05 mg/L Cu showed increased whole-body Cu levels. The reason that, observed endocrine stress response to Cu may have been triggered directly by Cu entering the embryo; on the other hand, the concomitant decreased whole-body Ca levels indicate mineral disturbances likely as a result of deleterious effects of Cu on the chloride cells[21] and such a disturbance may have elicited a stress response indirectly.[43]

Table 4. Estimated LC Values and 95% confidence limits for larvae 48, 72 and 96 hours exposed to copper

Toxicity expression	*Concentration mg/L Cu (II) (lower 95%–upper 95%)*		
	48 hours	*72 hours*	*96 hours*
LC_1	0.014 (0.002–0.033)	0.006 (0.002–0.011)	0.005 (0.02–0.010)
LC_5	0.033 (0.008–0.063)	0.013 (0.005–0.021)	0.011 (0.005–0.018)
LC_{10}	0.052 (0.017–0.089)	0.019 (0.009–0.029)	0.016 (0.008–0.025)
LC_{15}	0.071 (0.028–0.113)	0.025 (0.014–0.037)	0.021 (0.012–0.031)
LC_{50}	0.261 (0.182–0.375)	0.083 (0.062–0.017)	0.064 (0.048–0.081)
LC_{85}	0.963 (0.606–2.400)	0.276 (0.207–0.418)	0.195 (0.151–0.280)
LC_{90}	1.311 (0.770–3.892)	0.366 (0.264–0.601)	0.254 (0.190–0.391)
LC_{95}	2.071 (1.091–8.034)	0.557 (0.375–1.041)	0.374 (0.264–0.649)
LC_{99}	4.881 (2.065–31.704)	1.222 (0.715–2.963)	0.778 (0.481–1.711)
χ^2 for heterogeneity	4.312	7.175	3.637

Tabular $\chi^2 = 9.488$ at 0.05 confidence level.
Values in underlined style correspond with estimated concentrations higher of the upper experimental concentration of the test (> 0.5 mg/L).

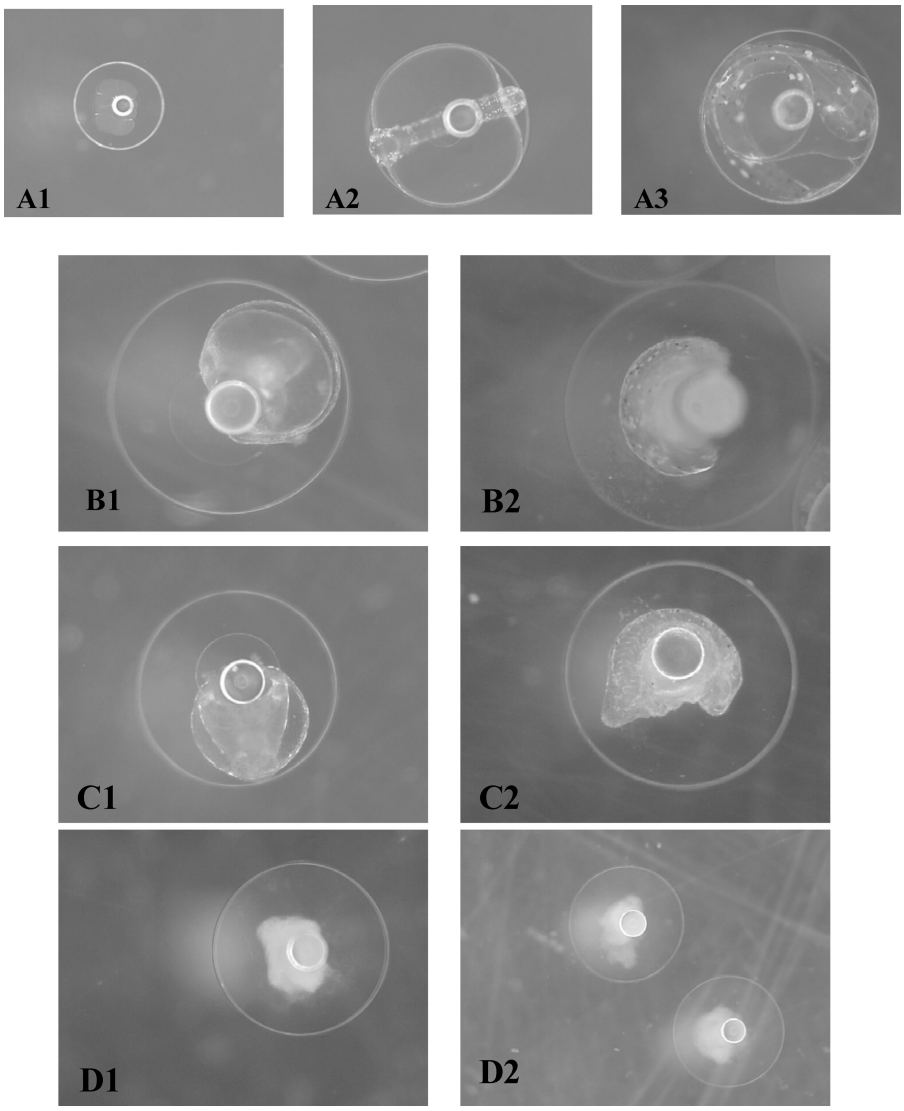

Fig. 2. Digital microphotographies of *S. aurata* embryos exposed to copper. A) Control embryos. (A1) 2-h after spawning embryo (×25). (A2) 24-h after spawning embryo (×26). (A3) 48-h after spawning embryo (x 28). (B) Embryos exposed to 0.025 mg/L of copper: (B1) 24-h exposure (×31). (B2) 48-h exposure (×31). (C) Embryos exposed to 0.050 mg/L: (C1) 24-h exposure (×31). (C2) 48-h exposure (×31). (D) Embryos exposed to 0.075 mg/L of copper: (D1) 24-h exposure (×31). (D2) 48-h exposure (×31).

The effects of metals on pigmentation have been observed in sebrafish, *Danio rerio* exposed to lead,[44] in *Clarias gariepinus*[45] and *Cyprinus carpio* exposed to copper.[46] The suppression of the alpha-melanocyte-stimulating hormone (α-MSH) response by copper in the developing carp may relate to an action during the early development of its MSH-cells.[37] Nguyen and Janssen,[45] observed a clear concentration-response relationship between the percentage of larvae with affected pigmentation and the Cu concentrations. In these larvae, the melanophores were more clumped in comparison with those in the control larvae. Reduced pigmentation was significant at $\geq$ 0.6 mg/L Cu.

Note on histopathological manifestations

Histopathological manifestations in different tissues of gilthead seabream larvae exposed to various concentrations of copper at 24 and 96 hours exposure are show in Tables 5, 6 and 7. We can observe the major alteration in the digestive tissue (Table 5). At 0.25 mg/L copper and 24 hours exposure, the mucous cells present a severe alteration with an increment of exudates. A great cellular disorganization in the kidney hematopoietic tissue is observed in larvae exposed to 0.075 mg/L copper (Table 7), the alterations in this tissue have been practically inexistent to lesser concentrations. The lesion grade of the hepatic alterations was

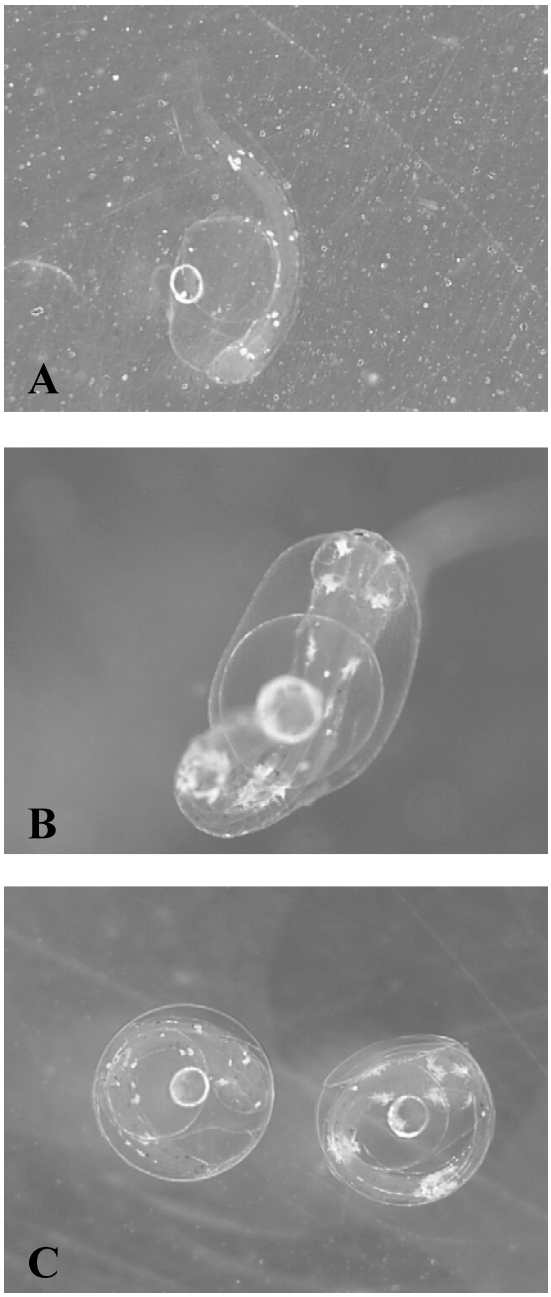

Fig. 3. Development stage of *S. aurata* embryos exposed to copper at 72-h exposures. (A) 0.025 mg/L of copper (×25). (B) 0.050 mg/L of copper (×31). (C) 0.075 mg/L de of copper (×28).

not elevated (Table 6). Krishnani et al.[39] reported in *Lates calcarifer* exposed to 0.1–3.0 ppm copper, an initiation of honey-comb vacuolization in the hepatic tissue, melinization in kidney tissue and depletion of cells in the tubular interstitium. Gardner and LaRoche[48] reported necrosis of epithelial lining of various organs of kill fish (*F. heteroclitus*) including those in sensory systems due to short exposure to copper. Baker[49] reported necrosis of kidney hematopoietic tissue in winter flounder (*Pseudopleuronectes americanus*).

Table 5. Digestive tissue changes of *S. Aurata* larvae following exposure to different copper concentrations

Lesions	*Time exposure (hours)*	*Copper concentration (mg/L)*	
		0.025	*0.075*
Hypertrophy	24	++	++
	96	+++	+++
Exudates	24	+++	+++
	96	+++	+++
Epithelium desquamation	24	++	++
	96	+++	+++
Glandular alteration	24	+	+
	96	+	++
Picnosis	24	+	+
	96	+	++
Necrosis	24	+	+
	96	++	++

The number of plus symbols is proportional to the assessed damage. Lesion grade: (−) absent, (+) light, (++) moderated, (+ + +) high.

Table 6. Hepatic tissue changes of *S. Aurata* larvae following exposure to different copper concentrations

Lesions	*Time exposure (hours)*	*Copper concentration (mg/L)*	
		0.025	*0.075*
Necrosis	24	−	+
	96	+	+
Cellular disorganization	24	+	++
	96	++	++

The number of plus symbols is proportional to the assessed damage. Lesion grade: (−) absent, (+) light, (++) moderated, (+ + +) high.

Table 7. Renal tissue changes of *S. Aurata* larvae following exposure to different copper concentrations

Lesions	*Time exposure (hours)*	*Copper concentration*	
		0.025 mg/l	*0.075 mg/l*
Necrosis	24	−	+
	96	+	++
Hematopoietic disorganization	24	+	++
	96	+	+++
Picnosis	24	-	+
	96	+	++

The number of plus symbols is proportional to the assessed damage. Lesion grade: (−) absent,(+) light, (++) moderated, (+ + +) high.

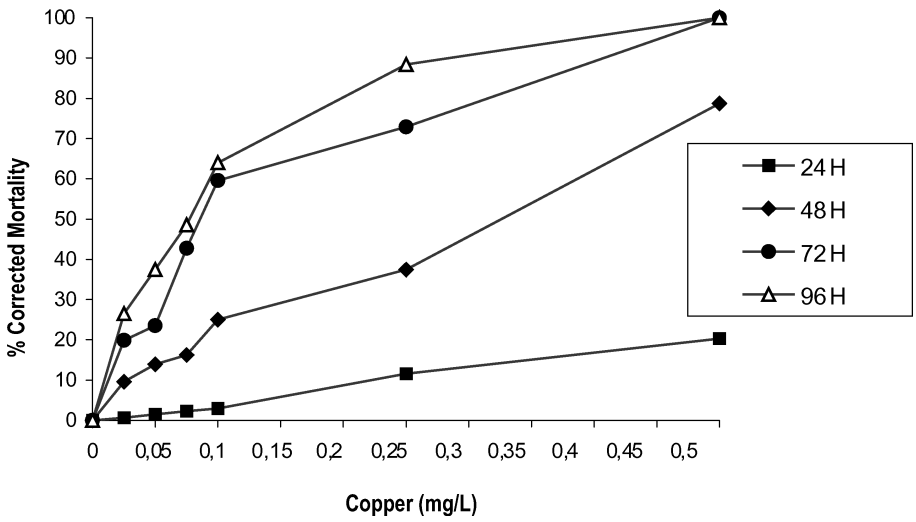

Fig. 4. Relationship between copper concentration and percentage of corrected mortality in *Sparus aurata* larvae.

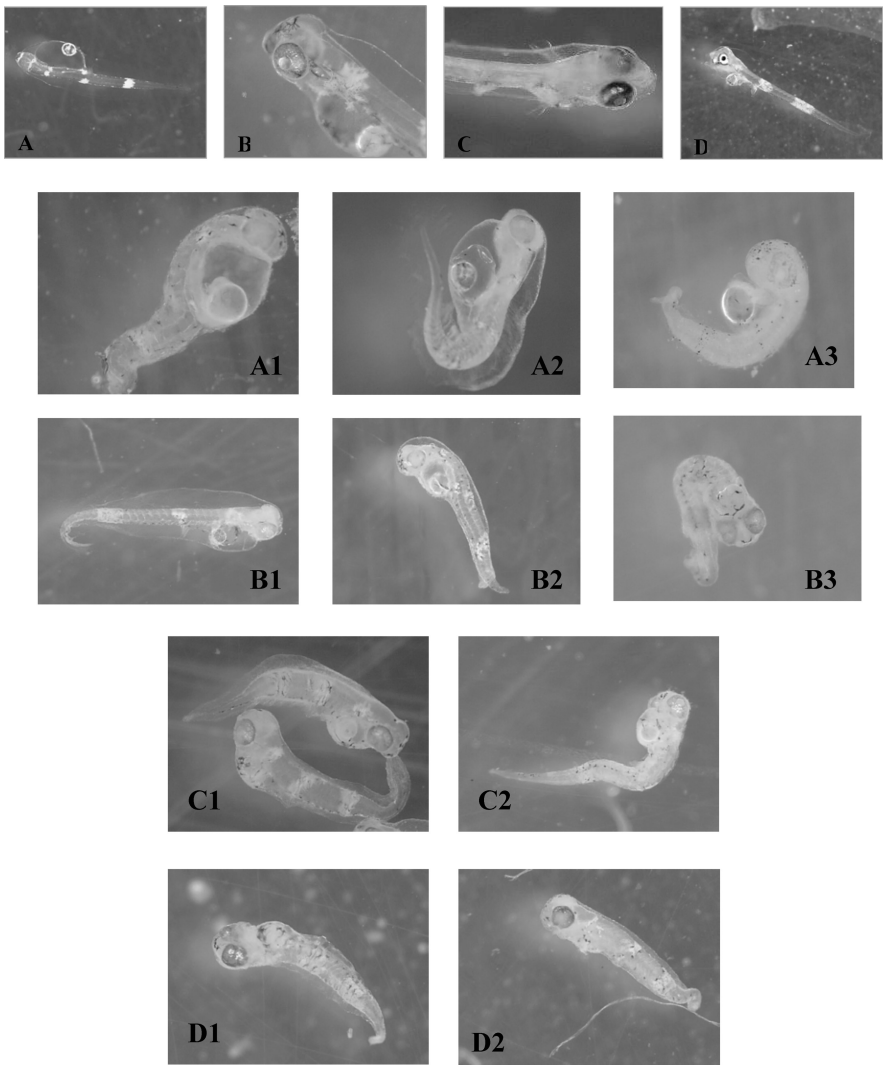

Fig. 5. Morphological alterations in several development stages of *S. aurata* larvae exposed to copper. (A) 24-h posthatching. (A1) 0.1 mg/L of copper (× 33). (A2) 0.25 mg/L of copper (× 26). (A3) 0.5 mg/L of copper (× 31). (B) 48-h posthatching. (B1) 0.1 mg/L of copper (× 17). (B2) 0.25 mg/L of copper (× 36). (B3) 0.5 mg/L of copper (× 25). (C) 72-h posthatching. (C1) 0.1 mg/L of copper (× 24). (C2) 0.25 mg/L of copper (× 22). (D) 96-h posthatching (D1) 0.1 mg/L of copper (× 23). (D2) 0.25 mg/L of copper (× 22).

Toxicity results and regional water quality criteria of copper (II)

Andalusia is a region located in the south of Spain, between the Atlantic Ocean and the Mediterranean Sea. The regional government of Andalusia has approved two water quality objectives (WQO) for copper (II) (Order 14.02.1997). For normal, open and non-enclosure waters or waters of a special ecological value, the WQO is 20 μg/L, while for limited waters, estuaries, bays and any other enclosure waters (hatchery zones of this fish) the WQO is 40 μg/L. The toxic concentrations obtained in this work demonstrate that the criteria for limited waters do not protect the early stages of *Sparus aurata*. Toxicity results in embryos (Table 2) show that the WQC could cause a mortality of about 15% after 48 hours of exposure to surface water with this concentration of copper. In larvae, Table 4 shows approximately the same percentage of effect for exposure times of 72 and 96 hours.

From this work and due to the importance of *Sparus aurata* in the Andalusia littoral, we recommended the revision of the actual WQC and the election of new objectives according to the sensibility of this species.

Conclusion

We conclude that

(1) *Sparus aurata* is a suitable species to test the toxicity of chemicals to marine organisms due to the short duration of *S. aurata* embryo-larval assay and its sensitivity.
(2) *Sparus aurata* embryos were more sensible to copper than larvae.
(3) The test with embryos is highly recommendable because it has an easy procedure, and very rapid and relevant results.

We recommended the toxicity test with early stages of this fish to develop water quality criteria in coastal waters. Future work in order to complete these results includes the realization of bioassays to determine copper adsorption by the chorion.

Acknowledgments

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References

[1] USEPA. *United States Environmental Protection Agency.* Ambient water quality criteria for copper (1984). Office of Water, Regulations and Standards, Criteria and Standards Division. Washington, DC 1985.
[2] EIFAC. Report on copper and freshwater fish. In: *Water Quality Criteria for European Freshwater Fish*. EIFAC Technical Papers, 1976.
[3] Knezovich, J.P.; Harrison, F.L.; Tucker, J.S. The influence of organic chelators on the toxicity of copper to embryos of the Pacific oyster, *Crassostrea gigas*. Arch. Environ. Contam. Toxicol. **1981**, *10*, 241–249.
[4] Effler, S.W.; Linen, S.; Field, S.D.; Tong-Nork, T.; Hale, F.; Meyer, M.; Quirck, M. Whole lake responses to low level copper sulphate treatment. Water Res. **1980**, *14*, 1489–1499.
[5] Carbonell, G.; Tarazona, J.V. A proposed method to diagnose acute copper poisoning in cultured rainbow trout, *Oncorynchus mykiss*. The Science of the Total Environment, Supplement 1993. Proceedings of the Second European Conference on Ecotoxicology. Elsevier Science Publishers B.V.: Amsterdam, 1993; 1329–1334.
[6] Lewis, A.G.; Cave, W.R. The biological importance of copper in oceans and estuaries. Oceanogr. Mar. Biol. Ann. Rev. **1982**, *20*, 471–695.
[7] Lewis, A.G.; Metaxas, A. Concentrations of total dissolved copper in and near copper-treated salmon net pen. Aquaculture **1991**, *99*, 269–276.
[8] Peterson, L.K.; D'auria, J.M.; McKeown, B.A.; Moore, K.; Shum, M. Copper levels in the muscle and liver tissue of farmed chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture **1991**, *99*, 105–115.
[9] Sorense, E.M. Cadmium. In *Metal Poisoning in Fish*. CRC Press: Boca Raton, FL, 1991; 175–234.
[10] Dethloff, G.M.; Schlenk, D.; Jonathan, T.H.; Bailey, H.C. Alteration in physiological parameters of rainbow trout (*Oncorhynchus mykiss*) with exposure to copper and copper/zinc mixtures. Ecotoxicol. Environ. Safety. **1999**, *42*, 253–254.
[11] Burridge, L.E.; Zitko, V. Lethality of copper sulfate and copper-treated nets to juvenile haddock, *Melanogrammus aeglefinus* L. Bull. Environ. Contam. Toxicol. **2002**, *69* (3), 378–383.
[12] Grosell, M.; Wood, C.M.; Walsh, P.J. Copper homeostasis and toxicity in the elasmobranch *Raja erinacea* and the teleost *Myoxocephalus octodecemspinosus* during exposure to elevated water-borne copper. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. **2003**, *135*(2), 179–190.
[13] Grosell, M.; McDonald, M.D.; Walsh, P.J.; Wood, C.M. Effects of prolonged copper exposure in the marine gulf toadfish (*Opsanus beta*) II: copper accumulation, drinking rate and Na/K ATPase activity in osmoregulatory tissues. Aquat. Toxicol. **2004**, *24*; 68(3), 263–275.
[14] Gravenmier, J.J.; Johnston, D.W.; Santone, R.C.; Arnold, W.R. Acute toxicity of copper to the threespine stickleback Gasterosteus aculeatus. Environ. Toxicol. **2005**, *20*(2), 150–159.
[15] Romani, R.; Antognelli, C.; Baldracchini, F.; De Santis, A.; Isani, G.; Giovannini, E.; Rosi, G. Increased acetylcholinesterase activities in specimens of *Sparus aurata* exposed to sublethal copper concentrations. Chem. Biol. Interact. **2003**, *15*, 145(3), 321–329.
[16] Isani, G.; Andreani, G.; Monari, M.; Carpene, E. Metal concentrations (Cu, Zn and Cd) and metallothionein expression in *Sparus aurata* exposed to waterborne copper. J. Trace. Elem. Med. Biol. **2003**, *17* Suppl 1, 17–23.
[17] Lauren, D.J.; McDonald, D.G. Acclimation to copper by rainbow trout, *Salmo gairdneri*: Physiology. Can. J. Fish. Aquat. Sci. **1987**, *44*, 99–104.
[18] Taylor, E.W.; Beaumont, M.W.; Butler, P.J.; Mair, J.; Mujallid, M.S.I. Lethal and sublethal effects of copper upon fish: a role for ammonia toxicity. Taylor EW (ed). Toxicology of aquatic pollution: physiological, cellular and molecular approaches. Cambridge University Press, Cambridge, United Kingdom: 1996; 85–113.
[19] Nussey, G.; Van Vuren, J.H.J.; Du Preez, H.H. Acute toxicity tests of copper on juvenile Mozambique tilapia, *Oreochromis mossambicus*

(Cichlidae), at different temperatures. S. Afr. J. Wild. Res. **1996**, *26*, 47–55.

[20] Svecivicius, G.; Vosyliene, M.Z. Acute toxicity of copper to common freshwater fishes of Lithuania. Ekol. Ehkol. Ecol. **1996**, *2*, 17–21.

[21] Li, J.; Quabius, E.S.; Wendelaar Bonga, S.E.; Flik, G.; Lock, R.A.C. Effects of water-borne copper on brachial chloride cells and Na^+/K^+-ATPase activities in Mozambique tilapia (*Oreochromis mossambicus*). Aquat. Toxicol. **1998**, *43*, 1–11.

[22] Priya, K.; Dinesh, K.P.B.; Ramesh, M.; Manavalaramanujam, R. Impact of copper sulphate toxicity on plasma electrolytes of a freshwater teleost fish, *Cyprinus carpiovar communis.* Ind. J. Fish. **1999**, *46*, 173–177.

[23] Perschbacher, P.W.; Wurts, W.A. Effects of calcium and magnesium hardness on acute copper toxicity to juvenile channel catfish, *Ictalurus punctatus*. Aquaculture **1999**, *172*, 275–280.

[24] McGeer, J.Z.; Szebedinszky, C.; McDonald, D.G.; Wood, C.M. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout. I: Ion-regulatory disturbance and metabolic costs. Aquat. Toxicol. **2000**, *50*, 231–243.

[25] Suau, P.; López, J. Contribución al estudio de la dorada *Sparus aurata* L. Investigaciones Pesqueras. 1976, 40, 169–199.

[26] Bauchet, M.L.; Hureau, J.C. Sparidae. In: Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C., Nielsen, J., Tortonese, E., Eds. Fishes of the North-Eastern Atlantic and the Mediterranean. Vol. II. UNESCO, Bungary: 1986; 883–907.

[27] Arias, A.; Drake, P. Estados juveniles de la ictiofauna en los caños de las salinas de la Bahía de Cádiz. Instituto de Ciencias Marinas de Andalucía: Cádiz, 1990.

[28] FAO. *The World State of Fishery and Aquaculture*. Roma, Italy, 1997.

[29] Del Valls, T.A.; Blasco, J.; Sarasquete, C.; Forja, J.M.; Gomez-Parra, A. Evaluation of heavy metal sediment toxicity in littoral ecosystems using juveniles of the fish *Sparus aurata*. Ecotoxicol. Environ. Saf. **1998**, *41*, 157–167.

[30] Hampel, M.; Blasco, J. Toxicity of linear alkylbencene sulfonate and one long-chain degradation intermediate, sulfophenyl carboxylic acid on early life-stages of seabream (*Sparus aurata*). Ecotoxicol. Environm. Saf. **2002**, *51*, 53–59.

[31] Dimitriou, P.; Castritsi-Catharios, J.; Miliou, H. Acute toxicity effects of tributyltin chloride and triphenyltin chloride on gilthead seabream, *Sparus aurata* L., embryos. Ecotoxicol. Environ. Saf. **2003**, *54*(1), 30–35.

[32] Arufe, M.I.; Arellano, J.; Moreno, M.J.; Sarasquete, C. Comparative toxic effects of formulated simazine on *Vibrio fischery* and gilthead seabream (*Sparus aurata* L.) larvae. Chemosphere **2004**, *57*, 1725–1732.

[33] Arufe, M.I.; Arellano, J.; Moreno, M.J.; Sarasquete, C. Toxicity of a commercial herbicide containing terbutryn and triasulfuron to seabream (*Sparus aurata* L.) larvae: a comparison with the microtox test. Ecotoxicol. Environ. Saf. **2004**, *59*, 209–216.

[34] USEPA. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. Office of Water; Washington, DC, 2002.

[35] Alderdice, D.F. Osmotic and ionic regulation in teleost eggs and larvae. In: Hoar, W.S., Randall, D.J. (Eds.), *Fish Physiology*. Academic Press; San Diego, 1988, 163–251.

[36] Hampel, M.; Blasco, J. Toxicity of linear alkylbenzene sulfonate and one long-chain degradation intermediate, sulfophenyl carboxilic Acid on early life-stages of sea bream (*Sparus aurata*). Ecotoxicol. Environ. Saf. **2002**, *51*, 53–59.

[37] Flik, G.; Stouthart, X.J.; Spanings, F.A.; Lock, R.A.; Fenwick, J.C.; Wendelaar Bonga, S.E. Stress response to waterborne Cu during early stages of carp, *Cyprinus carpio*. Aquat. Toxicol. **2002**, *56*(3), 167–176.

[38] Hamilton, S.J.; Buhl, K.J. Hazard evaluation of inorganics, singly and in mixtures, to flannelmouth sucker *Catostomus latipinnis* in the San Juan River, New Mexico. Ecotoxicol. Environ. Saf. **1997**, *38*, 296–308.

[39] Krishnani, K.K.; Azad, I.; Kailasam, M.; Thirunavukkarasu, A.R.; Gupta, B.P.; Joseph, K.O.; Muralidhar, M.; Abraham, M. Acute toxicity of some heavy metals to *Lates calcarifer* fry with a note on its histopathological manifestations. J. Environ. Sci. Health Pt. A. **2003**, *38*(4), 645–655.

[40] Nguyen, L.T.H.; Janssen, C.R. Embryo-larval toxicity tests with the african catfish (*Clarias gariepinus*): Comparative sensitivity of endpoints. Arch. Environ. Contam. Toxicol. **2002**, *42*, 256–262.

[41] Granmo, A.; Ekelund, R.; Sneli, J.A.; Berggren, M.; Svavarson, J.Effects of antifouling paint components (TBTO, copper and triazine) on the early development of embryos in cod (*Gadus morhua* L.). Mar. Poll. Bull. **2002**, *44*, 1142–1148.

[42] Thorsen, A.; Fyhn, H.J. Final oocyte maturation in vivo and in vitro in marine fishes with pelagic eggs, yolk protein hydrolysis and free amino acid content. J. Fish Biol. **1996**, *48*, 1195–1209.

[43] Wendelaar Bonga, S.E. The stress response in fish. Physiology **1997**, *77*, 591–625.

[44] Ozoh, P.T.E.. Effect of lead on pigment pattern formation in zebrafish (*Brachydanio rerio*). Bull. Environ. Contam. Toxicol. **1980**, *24*, 276–282.

[45] Nguyen, L.T.; Janssen, C.R. Comparative sensitivity of embryo-larval toxicity assays with African catfish (*Clarias gariepinus*) and zebra fish (*Danio rerio*). Environ. Toxicol. **2001**, *16*(6), 566–571.

[46] Iger, Y.; Lock, R.A.C.; Jenner, S.E.; Wendelaar Bonga, S.E. Cellular responses in the skin of carp (*Cyprinus carpio*) exposed to copper. Aquat. Toxicol. **1994**, *29*, 49–64.

[47] Roberts, R.J. *Fish Pathology*, 2nd Ed.; Baillier Tindal: London, 1989.

[48] Gardner, G.R.; LaRoche, G. Copper induced lesions in estuarine teleosts. J. Fish. Res. Board. Can. **1973**, *30*, 363–368.

[49] Baker, J.T.P. Histological and electron microscopical observations on copper poisoning in the winter flounder (*Pseudopleuronectes americanus*). J. Fish Res. Board. Can. **1969**, *26*, 2785–2793.