



## **European Sea Bass (*Dicentrarchus labrax* L. 1758) As a Sentinel Species in Europe to Study the Effects of Contaminants**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors SB and DC planned and organized all the experimental activities of the project "Inclusion of the European sea bass (*Dicentrarchus labrax* L. 1758) among species recommended for testing in the OECD Guidelines for testing of chemicals". Authors DC, AP, CM, ER, FC, FS, MC, VB and AP performed the acute toxicity tests with sea bass. Authors DC and SB wrote the manuscript. Authors AP, FS, MC and CM contributed to the paper preparation. All authors read and approved the final manuscript*

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### **ABSTRACT**

**Aim:** The current study was designed to evaluate whether European Sea Bass (*Dicentrarchus labrax* L. 1758) can be used as a sentinel species to test contaminants. For this aim the acute lethality (96 h) of anionic surfactant sodium dodecyl sulfate (SDS) on sea bass juveniles at two different water salinity percentages (20‰ and 5 ‰) were tested in order to assess how the water salinity may affect the sensitivity of the sea bass to SDS. A comparison with results from scientific literature especially freshwater species was also reported.

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**Study Design:** Five different sized fish groups (from 0.068 g to 1.42 g) were assessed in 96-h semi-static tests according to OECD guideline n. 203 (1992). The average 96h-LC<sub>50</sub> (plus 95% confidence interval) values were calculated using SDS nominal concentrations.

**Results:** At a percentage of salinity of 20‰, the 96h-LC<sub>50</sub> values increased from 5.76 mg/l to 9.50 mg/l increasing the size of juveniles as well. SDS at a salinity of 5‰, was always found to be significantly less toxic (96h-LC<sub>50</sub>: from 13.50 mg/l to 14.87 mg/l) independently of fish size.

**Conclusion:** The results of this study show that the characteristics of *D. labrax* make this species useful to provide information of toxicity in both seawater and freshwater. Its ease of maintenance and testing under laboratory conditions, and its broad euryhalinity confirm the convenience of use it as a sentinel species for detecting environmental impact and as a rule in monitoring studies. A comparison with other Italian and international data on sea bass is reported.

*Keywords:* Anionic surfactant; SDS; acute toxicity; European sea bass (*Dicentrarchus labrax*); salinity.

## 1. INTRODUCTION

Sea bass is a perciform teleost fish belonging to the Moronidae family and the genus *Dicentrarchus* (<http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>).

The species *Dicentrarchus labrax* L. 1758, named European or common sea bass, is primarily a marine fish, but it is also found in brackish and freshwater [1]. It was the first marine non-salmonid species to be commercially cultured in Europe and, actually, it is the one of the most important commercial fish widely reared in the Mediterranean areas: Greece, Turkey, Italy, Spain, Croatia and Egypt are the biggest producers [2].

Usually the adult sea bass is a highly eurythermal and euryhaline species. In Winter, it lives in waters at 5-6°C in the lagoons of the northern Mediterranean. In Summer, it is abundant in waters where temperatures exceed 30°C. *D. labrax* can also survive in a wide variety of environmental salinity levels (0.5 – 35‰) [3,4].

It is well known that metabolism, development and growth of teleost fishes are controlled by internal factors (CNS, endocrinological and neuroendocrinological systems), but they are also highly dependent on environmental conditions [5]. Many studies have reported that water salinity is one of the determining factors (together with temperature and photoperiod) which directly acts through receptors to increase or decrease the growth and the development of fish [6].

For the purpose of this study, *D. labrax* was selected because of its ease of culture and maintenance under laboratory conditions. It is a well-known species, frequently used to

investigate the toxicity of various classes of chemicals [6-18] and also acts as a sentinel species (i.e. indicator species or bioindicator species) on monitoring studies [19-22].

*Dicentrarchus labrax* has extensively been used in Italy for regulatory evaluation of ecotoxicity. At least three important national projects concerning the sea bass were carried out. The first entitled "Priority Substances: Fish (*D. labrax*, *Cyprinus carpio*) as target organisms in toxicity, bioaccumulation and genotoxicity tests". It aimed at the toxicological assessment of dangerous chemicals recommended by the Italian Legislative Decree 152/2006 (Italian transposition of WFD 2000/60/CE) [23]. The second project entitled "Oil dispersants: toxicity testing with marine organisms (Algae, Crustaceans and Fish)". The fish species selected in this study (*D. labrax* and *Sparus aurata*) were included in the Italian Decree of the Environment Ministry (25 February 2011, Official Journal n. 74, 31 March 2011 - Supplement n. 87) concerning the procedures for assessing suitability of oil absorbent and dispersant products to be employed in marine environment, in order to minimize the impact of oil spills [24].

The latter project organized by UNICHIM (a National no-profit organization associated to the Italian Authority for Standardization, UNI) aimed to validate the guideline n° 203 of the Organisation for Economic Co-operation and Development (OECD) by using *D. Labrax* [25,26]

Furthermore, in Italy, the sea bass at different life stages (eggs, larvae, post-larvae and juveniles) was preferably used for testing various classes of chemicals [27-30].

Recently, a proposal concerning the "Inclusion of the European sea bass (*D. labrax* L.1758) among species recommended for testing in the

OECD Guidelines for testing of chemicals n° 203, 212, 215, 305" was presented by Italy at the 25<sup>th</sup> Meeting of the Working Group of National Coordinators of the Test Guidelines Programme (WNT 25) held from 9 to 11 April 2013. OECD Members highlighted the lack of representativeness of fish species in the toxicity testing guidelines (TG) as far as the Mediterranean Sea is concerned. They proposed to Italy to develop a Guidance Document (GD) on the use of the European sea bass in fish toxicity and bioaccumulation tests.

The quality of test organism is key to successful conduct of fish test and species selection should consider a number of different criteria including: size, ease of maintenance, convenience for testing, relevant economic biological or ecological factors, known sensitivity, pre-existing data, animal welfare, availability of cultured, as opposed, to field-collected organisms as well as national or regional preferences [31]. *Dicentrarchus labrax*'s compliance to most of these criteria was clearly recognized. The main strengths for using this species are its wide availability at every stage of the reproductive cycle in many countries of the Mediterranean Basin (Italy, Spain, Turkey, Greece, France and North Africa), its ease of maintenance and testing under laboratory conditions, and its broad euryhalinity. On the other hand, there are not any recommended estuarine and marine fish species in the acute (OECD TG 203), prolonged (OECD TG 204) [32] and juvenile growth test (OECD TG 215) [33]. Nevertheless, the OECD test guidelines present no restrictions in the choice of fish test species, provided that the alternative species fulfils the selection criteria mentioned above and the rationale for the choice is well documented.

Surfactants are economically important chemicals which are widely used in household cleaning detergents, personal care products, textiles, paints, pharmaceuticals, cosmetics, etc. They and their products are often discharged directly to waters or into sewage treatment plants and then dispersed into the environment. Due to their widespread use and their presence in the environment, a lot of studies were carried out to demonstrate that surfactants are capable of causing important adverse effects to the aquatic and terrestrial life [34].

Surfactants are amphiphilic compounds capable of reducing the surface tension of water by forming a monolayer at the air-water interface.

They are water-soluble in the micro- to millimolar range above which they self-associate to micelles, bilayer vesicles or other aggregates [35].

The sodium dodecyl sulfate ( $C_{12}H_{25}NaO_4S$  - SDS), also known as sodium lauryl sulfate, belongs to the class of anionic surfactants (fatty alcohol sulphates, AS). SDS exerts a low logP (partition coefficient octanol/water) and logHCl (partition coefficient water/air) value and is miscible in water (water solubility is 100g/l). Because of this, it can be easily handled and hampering sorption to exposure vessels or evaporation can be excluded [36].

The current study was designed to evaluate the acute lethality (96h) of anionic surfactant SDS on sea bass juveniles. Two different water salinity percentages (20‰ and 5‰) were tested in order to assess how the water salinity may affect the sensitivity of the sea bass to SDS. A comparison with SDS results from scientific literature was also reported.

## 2. MATERIALS AND METHODS

### 2.1 Test Chemical

Sodium Dodecyl Sulfate (CAS N° 151-21-3; EINECS: 205-788-1) Panreac PA-ACS (99% purity) was purchased from Nova Chimica (batch n°367414).

### 2.2 Test Fish

*Dicentrarchus labrax* juveniles were supplied by two different salt water fish farms: Ca' Zuliani Lagoon Fishing Farm (Porto Tolle, Rovigo – Veneto region, northern Italy) and Caldoli Fishing Farm (Lesina, Foggia – Puglia region, southern Italy). All fishes were acclimated for 14 days to laboratory conditions in breeding tanks equipped with mechanical and biological filter and a recirculating pump with capacity of 1000 liter/hour at the same conditions of the origin fishing farm. Breeding tanks (300 liter capacity) were filled with artificial seawater, fish loading was approximately 0.5-0.6 g/l. Water temperature had been keeping at  $20\pm 1^\circ C$  in continuous artificial aeration; oxygen concentration was between 7 to 9 mg/l and pH ranged from 7.2 to 8.5. After 48h we started to check the mortality for seven days; the mortality was less than 5% then the batch was accepted for testing. The juveniles sea bass were adapted

to salinity variation gradually in a time of 14 days (salt concentration was measured with Atago® salinometer, accuracy 0.1%). The fishes were regularly fed (about 2% by fish weight) with commercial fish food provided by the farmers with a suitable composition to their nutritional needs.

The concentration of the main catabolites,  $\text{NH}_4$ ,  $\text{NO}_2$  and  $\text{NO}_3$  (Salifert – Ammonia, Nitrite and Nitrate Prof-Test kit), the dissolved oxygen concentration (HI9143 portable Dissolved Oxygen Meter, Hanna Instruments) and the pH value of the water (HI9025C portable Waterproof pH meters, Hanna Instruments) were measured every day. Ammonia and nitrite are usually absent in the breeding water (nitrate concentration is acceptable until 30 mg/l).

The fish specimens were divided in five different groups according to their size; the mean values of weight (g) and total length (cm) are shown in Table 1.

The juvenile sea bass specimens have been led from 5‰ to 20‰ of salt water gradually at least fifteen days before they are used.

### 2.3 Acute Toxicity Tests

European sea bass, *D. labrax*, were tested in a 96-h semistatic test according to OECD guideline n° 203 (1992). Seven independent toxicity tests

were conducted in a room with controlled temperature and photoperiod (16hL:8hD). Based on the fish sizes, different test chambers were used (test organism loading not exceeding 1 g/l) in the tests. The quality of water was monitored on a daily basis for the duration of the tests by measuring water dissolved oxygen, pH and temperature. No food was provided to fish during the tests.

The nominal exposure concentrations used in the SDS definitive assays were selected according to a preliminary range-finding test, as they are shown in Table 2.

Nominal SDS concentrations were obtained by serial dilutions of a stock solution (100 mg/l) prepared in ultrapure water immediately before use.

The fatty alcohol sulphates such as SDS, are among the most rapidly biodegradable compounds in the environment. Steber et al., 1988 [37] reported for fatty alcohol sulphates a primary degradation of 95-98% in a 5 days period. SDS biodegradation was reported in Antarctic coastal waters with half lives of 160-460 hours [38]. In order to minimize disturbance of the test organisms and the degradation of SDS, test solutions were carefully renewed every 24 or 48h. A saltwater control was included in each experiment.

**Table 1. Features of fishes *Dicentrarchus labrax* (Moronidae) used in experimental procedures (in increasing order)**

Fish group	N° of specimens	W(g)	TL(cm)	Fishing farm
I	150	0.068±0.03	2.23±0.22	Ca Zuliani Lagon Fishing Farm, Rovigo, Italy
II	150	0.50±0.07	3.90±0.30	Caldoli Fishing Farm, Foggia, Italy
III	140	0.66±0.22	4.64±0.29	Caldoli Fishing Farm, Foggia, Italy
IV	140	0.94±0.34	4.79±0.52	Ca Zuliani Lagon Fishing Farm, Rovigo, Italy
V	140	1.42±0.05	5.40±0.07	Caldoli Fishing Farm, Foggia, Italy

Abbreviations: W: weight; TL: total length

**Table 2. Exposure conditions used in the sodium dodecyl sulfate acute toxicity testing on *Dicentrarchus labrax* (Moronidae)**

Expt	Fish group	Salinity (‰)	T (°C)	pH	Renewal	n°org/ chamber	R	Total n° fish	SDS nominal concentrations (mg/l)
1	I	20	20.0±0.7	8.5±0.1	every 48h	7	3	126	2.5 - 3.6 - 5.0 - 7.1 - 10
2	II	5	19.6±0.3	8.4±0.2	every 48h	7	3	126	10 - 16 - 25 - 40 - 63
3	III	20	19.5±1	8.4±0.2	every 48h	10	1	60	1 - 5 - 9 - 13 - 17
4	III	5	19.5±1	8.4±0.2	every 48h	10	1	60	5 - 9 - 13 - 17 - 21
5	IV	20	20.0±1	8.5±0.1	every 48h	7	3	126	2.5 - 5 - 10 - 20 - 40
6	V	5	20.5±1	8.5±0.1	every 24h	10	1	60	6 - 9 - 12 - 15 - 18
7	V	20	20.5±1	8.4±0.1	every 24h	10	1	60	3.5 - 5.5 - 7.5 - 9.5 - 11.5

Abbreviations: R: replicate

A summary of the exposure conditions used in each experiment is shown in Table 2.

Fish were inspected every 30 minutes for the first 8 hours and subsequently at 24, 48, 72 and 96h for mortality and other visible abnormalities (e.g. swimming behaviour, reduction of reflexes, respiratory function, etc.).

The fish were considered to be dead when no vitality signs, such as movement of gills, escape reaction and body movement could be observed.

All animal studies were conducted in accordance with national law (D.lgs 26/2014) and European Directive 2010/63/UE on the protection of animals used for scientific purposes.

### 2.4 Data Analysis

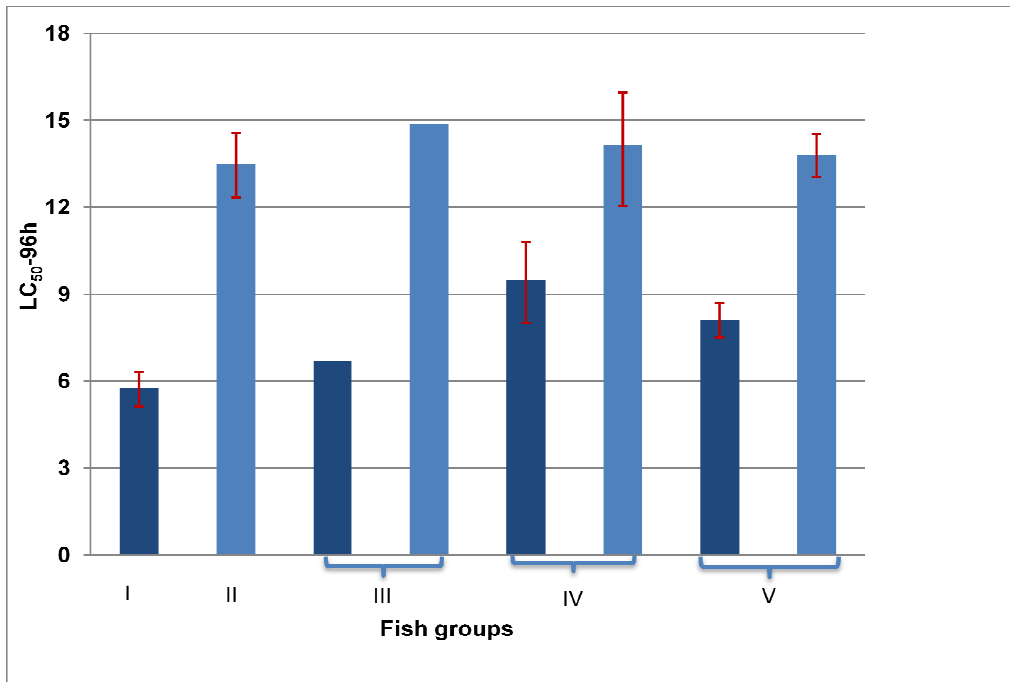
Results of the toxicity tests on SDS are reported as Median-Lethal-Concentration ( $LC_{50}$ ).  $LC_{50}$  values and their 95% confidence intervals were calculated by using Trimmed-Spearman-Kärber (TSK) method version 1.5 (TOXStat software package) [39].

### 3. RESULTS

All the acute toxicity tests performed with sea bass exposed to SDS were considered valid, since no mortality was observed in all of the control groups, the range of pH values was always lower than 1,0 pH unit and the oxygen concentrations were always higher than 60 per cent of the air saturation value during the test.

Sodium Dodecil Sulfate nominal concentrations were used to calculate the median lethal concentrations at 96h (96h- $LC_{50}$ ), and their 95% confidence intervals, at two different salinities (20‰ and 5‰). The results are shown in the Table 3.

The analysis aiming at confirming the SDS test concentrations were not performed during our sea bass acute toxicity tests. However, we conducted the tests with a renewal of SDS solutions every 24 and 48 hours. Results obtained with a 24h renewal (96h- $LC_{50}$ : 8.12 mg/l at salinity of 20‰ and 13.81 at salinity of 5‰) were comparable with those obtained with a 48h renewal (96h- $LC_{50}$ : 9.50 mg/l at salinity of 20‰ and 14.14 at salinity of 5‰).



**Fig. 1. Histogram of 96h- $LC_{50}$ 's obtained with sodium dodecyl sulfate at two different salinities 20‰ (dark column) and 5‰ (light column) on five different sized sea bass (*Dicentrarchus labrax*) groups**

**Table 3. Median Lethal Concentration (96h-LC<sub>50</sub>) of sodium dodecyl sulfate for *Dicentrarchus labrax* juveniles at two different salinities (20 and 5‰)**

Fish group	W (g)	TL (cm)	96 h-LC <sub>50</sub> (95% CI) mg/l	
			20 ‰	5 ‰
I	0.068±0.03	2.23±0.22	5.76 (5.20-6.40)	-
II	0.50±0.07	3.90±0.30	-	13.50 (12.43 – 14.67)
III	0.66±0.22	4.64±0.29	6.71 *	14.87 *
IV	0.94±0.34	4.79±0.52	9.50 (8.2-11.0)	14.14 (12.32-16.24)
V	1.42±0.05	5.40±0.07	8.12 (7.55-8.73)	13.81 (13.09-14.57)

Abbreviations: W: weight; TL: total length; CI: confidence interval;

Note: \*The 95% CI is not reliable because no SDS concentration was found to prove an intermediate toxicity

As shown in Fig. 1, the SDS at salinity of 5‰ was always found to be significantly less toxic for juvenile sea bass independently of the fish size. In the present study, the reduction of salinity to 5‰ resulted in a decreased toxicity of SDS for all exposed fish groups (96h-LC<sub>50</sub>: (II) 13.50 mg/l; (III) 14.87 mg/l; (IV) 14.14 mg/l; (V) 14.87 mg/l).

Significant differences were also seen (in Fig. 1) at a salinity of 20‰ among different fish groups. By raising the size of organisms from 2.23±0.22 cm (fish group I) to 4.79±0.52 cm (fish group V) the sensitivity of fish to SDS decreased (96h-LC<sub>50</sub>: 5.76 mg/l vs 9.50 mg/l). On the other hand, no significant differences in toxicity to SDS were found between fish groups IV and V, probably due to similar size of sea bass.

#### 4. DISCUSSION

The European sea bass was tested for chemical toxicity under many different conditions [40-47]. However, it is very important to understand how salinity might affect the toxicity of chemicals since euryhaline organisms can be employed in laboratory testing.

Some studies have demonstrated that sea bass is able to live in low salinity environments and to successfully adapt to freshwater under experimental conditions [48,49], even though in other studies mortality was recorded when fish were exposed to fresh water (<1‰) [50]. It was also found that sea bass juveniles have a low saline *preferendum* (15‰) which corresponds to the conditions this species encounter in the wild during their juvenile ecophase [51].

Moreover in order to examine the possible benefits of culturing sea bass at reduced salinities, Dendrinou and Thorpe [52] investigated the effects of various salinities (33, 30, 25, 20, 15, 10, 5‰) on growth and body composition in sea bass juveniles over a period of 1 year. They found out that all specimens were able to survive at reduced salinity percentages

(until 5‰), whereas for those kept in freshwater, mortality occurred within the first 20 days. At salinities ranging from 18‰ to 8‰, *D. labrax* show lower locomotion and less agility and swimming activity in the tanks, in order to reduce food energy consumption and attribute larger portions for osmoregulation [53]. As salinity decreases (from 30‰ to 20, 10 and 5‰) the sea bass, in resting conditions, reduces its metabolic rates [54].

The toxicity of many chemicals was reported to vary according to water salinity. A critical review which investigated the effects of salinity on the toxicity of various classes of inorganic and organic chemicals to aquatic biota showed that negative correlations (such as toxicity increases while salinity decreases) are more frequent (55%) than positive (18%) and no correlations (27%) are also possible. The increasing toxicity with decreasing salinity observed with most metals (such as cadmium, chromium, copper, mercury, nickel and zinc) is probably due to the greater bioavailability of the free metal ion (toxic form) at lower salinity conditions [51]. Lower salinity (15‰) seems to increase the solubility of polycyclic aromatic hydrocarbons (PAH) from the oil dispersants, so that the exposure to PAH can be up to 60-fold greater compared to water of full salinity (32‰). On the other hand, organophosphate and carbamate insecticides toxicity appeared to decrease with decreasing salinity [55].

In the past, SDS has been often used by Italian laboratories as reference toxicant for assessing the sensitivity of *D. labrax* as well as for monitoring the condition of fish stocks and laboratory environments. The SDS toxicity data on sea bass from scientific literature, reported in Table 4, are referred at different life stages of sea bass, time exposures and salinities (from 20 to 35‰). In all reported studies, the LC<sub>50</sub> values are based on nominal SDS exposure concentrations.

**Table 4. Summary of Italian toxicity studies with sodium dodecyl sulfate on *Dicentrarchus labrax* at different life stages and test conditions**

LS and age	Sea bass		Test conditions					Results		References		
	W(g)	TL(cm)	T(C)	Salinity (‰)	TP	Time exp	OECD TG	LC50 (mg/l)	±SD		95% CI (mg/l)	
E 70-80 hpf	-	-	20±0.5	35	S	24h	203	< 1.12	-	-	Roncarati et al. [46]	
L 30 d	0.005	0.011	20±0.5	20±1	S	24h	203	1.89	-	-	Roncarati et al. [46]	
L 30 d	-	-	20±1	30±1	S	24h	-	2.04	-	1.38-2.52	Cicero et al. [63]	
L 30 d	-	-	20±1	20±1	S	24h	203	2.90	-	2.64-3.18		
L 30 d	-	-	20±1	20±1	S	24h	203	2.66	-	1.99-3.55		
PL	0.06	-	20±0.5	15±1	S	24h	-	1.89	-	1.65-2.15	Spaggiari et al. [23]	
J	0.84	-	20±0.5	15±1	S	24h	-	5.78	-	-	Roncarati et al. [46]	
J	3.40	-	20±0.5	15±1	S	24h	-	9.85	-	-	Roncarati et al. [46]	
J 50 d	-	1.21±0.14	21±1	30±1	S	24h	203	10.72	-	-		
J 50 d	-	1.21±0.14	21±1	30±1	S	24h	203	2.93	0.52	-	Mariani et al. [59]	
J 70 d	-	1.73±0.15	21±1	30±1	S	48h	203	0.90	0.50	-		
J	-	5.75±0.2	20±1	20±1	R	24h	203	3.98	0.99	-	Mariani et al. [26]	
J	0.82±0.3	5.0±1.0	20±1	20±1	S	48h	203	3.87	1.03	-		
J	0.82±0.3	5.0±1.0	20±1	20±1	S	96h	203	7.28	0.47	-	Gelli et al. [56]	
J	0.82±0.3	5.0±1.0	20±1	20±1	S	96h	203	7.07	-	5.0-10.0	Mariani et al. [57]	
J	0.82±0.3	5.0±1.0	20±1	20±1	S	96h	203	7.88	-	7.10-8.19		
J	from 0.53±0.1 to 4.77±0.2	from 4.4±0.3 to 7.84±0.3	20±1	20±0.5	R	48h	96h	203	7.33	0.53	-	ICRAM-TAXAp-[24]

Abbreviations: CI: confidence interval; d: days; e-L: embryo-larvae; E: eggs; hpf.: hours post fertilization; J: juvenile; L: larvae; LS: life stage; P: Test Procedure; PL: post larvae; R: test with renewal of medium; S: static test; SD: standard deviation; TL: Total Length; W: weight. Note <sup>a</sup> Experiments were carried out by four laboratories with different batches of fish

**Table 5. Summary of toxicity data with sodium dodecyl sulfate on different marine/estuarine fish species according to the IUCLID (2000) database of existing chemicals of the European Chemicals Bureau**

<b>Fish species</b>	<b>LS</b>	<b>S(‰)</b>	<b>Time exposure</b>	<b>LC<sub>50</sub> (mg/l)</b>	<b>SD</b>	<b>95% CI</b>	<b>References</b>
<i>Cyprinodon variegatus</i>	L 7 d	32	7 d	2.9	-	1.8-3.4	Morrison et al. [65]
<i>Menidia berillina</i>	L 7 d	32	7 d	1.8	-	-	Morrison et al. [65]
<i>Menidia berillina</i>	L 28 d	20	96 h	1.48	-	1.35-1.63	Hemmer et al. [64]
<i>Menidia menidia</i>	-	10	96 h	2.8	-	2.55-2.98	Roberts et al. [60]
<i>Cyprinodon variegatus</i>	-	15	96 h	9.0	-	-	Anderson et al. [61]
<i>Cyprinodon variegatus</i>	-	10	96 h	4.1	-	3.83-4.47	Roberts et al. [60]
<i>Gasterosteus aculeatus</i>	-	20	96 h	0.51	-	-	McAuliffe CD, et al. [62]
				4.2	-	-	
<i>Atheronops affinis</i>	L 22 d	20	96 h	1.88	-	1.67-2.11	Hemmer et al. [64]
<i>Fundulus heteroclitus</i>	A	20	96 h	5.60	-	-	La Roche et al. [66]
				4.70	-	-	
<i>Fundulus similis</i>	-	15-20	96 h	4.50	-	-	Anderson et al. [61]
<i>Sparus aurata</i>	J	20	96 h	7.66	0.81	-	ICRAM Taxap <sup>a</sup> [24]

Abbreviations: A: adult; d: days; h: hour; J: juvenile; L: larvae; LS: Life Stage; S: salinity; SD: Standard Deviation; CI: Confidence Interval.

Note: <sup>a</sup>Experiments were carried out by four laboratories with 5 different batches of fish



The comparisons of results among different species should be considered with caution to avoid bias in the conclusions due to different conditions tested and possible confusing factors. Despite of a large quantity of SDS toxicity data on sea bass (Table 4), a stringent comparison with our data was not possible, because of so many different test conditions and life stages of fish were employed in the experiments. Our 96h-LC<sub>50</sub> values at salinity of 20‰ (9.50 and 8.12 mg/l) are comparable with those obtained from Gelli et al. [56], Mariani et al. [57] and ICRAM-TAXA, [24] with same sized sea bass juveniles. Furthermore, a positive correlation between toxicity and salinity might be suggested between the nominal 24h-LC<sub>50</sub> values at a salinity of 15‰ and those at a salinity of 30‰ [58] although the sizes of juveniles seem to be different.

It desirable that species be representative of the estuarine biota in sensitivity to toxic substances. In the present study, the reduction of salinity to 5‰ resulted in a decreased toxicity of SDS for all exposed fish groups. Moreover, these results confirmed our nominal 96h-LC<sub>50</sub> value of 13.4 mg/l (95% CI: 12.4-14.4 mg/l) obtained with *Oncorhynchus mykiss* exposed in similar test conditions (fish size: 3.16±1.04 g and 6.7±0.7 cm) (*data not published*) and nominal 96h-LC<sub>50</sub> values of 16.2 mg/l and 17.0 mg/l reported in the IUCLID database (2000) with *Poecilia reticulata* and *Pimephales promelas*. At a salinity of 5‰, *D. labrax* seems to show sensitivity to SDS which could be compared with freshwater fish species recommended by OECD TGs.

A summary of toxicity data with SDS on other marine fish species is reported in Table 5. Here again, we observed a great variability in the SDS LC<sub>50</sub> values among species and also within species.

On the whole, the toxicity data derived from marine fish species are scarcely comparable with data on *D. labrax* because of different test, life stage and exposure conditions. The sea bass larvae with nominal 24h-LC<sub>50</sub> values ranging from 1.89 to 2.90 mg/l [63] seem to have a higher sensitivity in comparison with larvae of *Menidia berillina*, *Cyprinodon variegatus* [64] and *Atherinops affinis* [65] which show nominal 96h-LC<sub>50</sub> values of 1.8 mg/l, 2.9 mg/l and 1.88 mg/l, respectively. On the other hand, the nominal 96h-LC<sub>50</sub> value of 7.66±0.81 mg/L found in *Sparus aurata* (ICRAM Taxap-2005) [24] is

comparable with those of sea bass juveniles which ranging from 6.71 and 9.50 mg/L (our data) and from 7.07 to 7.88 mg/L [56,57,24].

Lastly, under similar conditions of salinity (20‰) and life stage, the sea bass seems to be less sensitivity in comparison with mummichog (*Fundulus heteroclitus*) which shows nominal 96h-LC<sub>50</sub> values of 5.60 and 4.70 mg/l [66].

## 5. CONCLUSIONS

In this study *D. labrax* confirms its ease of culture and maintenance under laboratory conditions, it is a well-known species, frequently used to investigate the toxicity of various classes of chemicals.

As euryhaline species, is able to adapting at different salinity condition both in nature and in laboratory conditions. In our experimental conditions juveniles sea bass at 5‰ salinity have comparable sensibility of Rainbow trout (*O. mykiss*) exposed to SDS in a similar test conditions. In this test conditions *D. labrax* seems to show sensitivity to SDS which could be compared with freshwater fish species recommended by OECD TGs. On the other hand, *D. labrax* shows an increasing sensibility at 20‰ water salinity; this means that if we change water salinity we get a more sensitive response from *D. labrax*. This characteristic could be helpful in case of use *D. labrax* like sentinel species for detecting environmental impact, like, for example, in assessing changing on water temperature due to climate change [67,68], on temperature increase due to water discharge from power plant [69], or studying the impact of toxic substances on the marine life like dispersant products (Gorbi et al. [1]. Tornambé et al. [14]) or priority substances (Roméo et al. [45]. Abreu et al., [19]. Antunes et al. [8]. Gravato et al. [16]. Banni et al. [10]. Ameer et al. [20]), as requested by Water Framework Directive.

The results of this study show that the characteristics of juveniles and adults of *D. labrax* make this species useful to provide information of toxicity in both seawater and freshwater. Its ease of maintenance and testing under laboratory conditions, and its broad euryhalinity confirm the convenience of use it as a sentinel species for detecting environmental impact and as a rule in monitoring studies.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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