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Sublethal effects of linear alkylbenzene sulphonate on larvae of the seabream (*Sparus aurata*): histological approach

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Summary. Neonate (<24h) larvae of the seabream, *Sparus aurata*, were exposed to sublethal concentrations (0.1-1.0 mg·L^{-1}) of the anionic surfactant Linear Alkylbenzene Sulphonate (LAS) for 72h under laboratory conditions. The first histopathological changes, such as peri-yolk sac edema, were observed at concentrations of 0.2 mg·L^{-1}. Higher exposure concentrations provoked disorganisation of the nervous system, trunk musculature and trophoblastic sincitium as well as in the digestive epithelium. Immunohystochemical CYP1A analysis, however, was not shown to be an adequate indicator of sublethal effects produced by exposure to this type of anionic surfactant.

Key words: Linear alkybenzene sulphonate, Sublethal effects, Seabream, Histology, CAS Nº 68411-30-3

Introduction

Linear Alkylbenzene Sulphonate (LAS) is the most widely used anionic surfactant in household and cleaning products. During wastewater treatment, up to 99% of LAS are removed (ECOSOL, 2002), thus concentrations of this compound in the receiving environments should be generally below effective concentrations (NOEC: no observed effect concentration) reported for fish, invertebrates and algae (De Henau et al., 1986). In cases of untreated wastewater discharge, concentrations of LAS may reach higher concentrations at the impact zone. Downstream of the initial discharge site, dilution, dispersion, and biodegradation processes play an important role in the reduction of initial concentrations to lower, potentially sublethal levels that could provoke effects on behaviour, development, reproduction or survival of the organisms, and, possibly, their offspring. However, in combination with other factors such as environmental stress, these concentrations could be more dangerous.

Generally, it is recommendable that toxicity assays are carried out with the most sensitive developmental stage(s) of a certain organism in order to guarantee the survival of the individuals during their complete lifespan. Fish larvae and embryos are frequently used in toxicity testing, as these developmental stages are recognized to be the most sensitive in the life-cycle of a teleost (Korn and Rice, 1981; McKim, 1985) and extrapolation to a whole life-cycle toxicity test is possible as both have shown to be equivalent (McKim, 1977). This fact allows brief exposure times to have important advantages as toxicity tests can be carried out more easily and quickly while providing reduction of costs.

In fish, surfactants have shown to produce damage in the gills, skin and pharynx (Bardach et al., 1965; Brown et al., 1968). Additionally, these compounds may penetrate into the organism and exert adverse effects on the internal functions. The resulting damage is generally manifested by loss of orientation, tendency to swallow air, lethargy or death. Sublethal effects of LAS exposure include pathological changes in gills, intestine, swim bladder, kidney and eyes (Sarasquete et al., 1997). In fish fingerlings (*Cyprinus carpio*) exposed to sublethal concentrations of LAS, Misra et al., (1991) found alterations in the levels of glycogen, lactic acid, sialic acid, and acid and alkaline phosphatases in the gills, liver and kidney at concentrations of 0.005 mg·L^{-1}. Nevertheless, as early life-stages do not have completely developed organs, sublethal effects of LAS exposure in early life-stages may manifest themselves in a different way to that mentioned by these authors.

Apart from routine microscope observation in order to detect malformations in organs and internal structures by histomorphological techniques, the use of in vitro toxicity assays has increased in recent years, due to advantages such as sensitivity, small samples and controlled conditions. An increasingly utilized biomarker for the analysis of the effects produced by organic lipophilic compounds is the enzymatic complex "Cytochrome P450s (CYP)" (Cajaraville et al., 2000;

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Sarasquete and Segner, 2000; Ortiz-Delgado et al., 2002). The cytochrome P450-dependent mono-oxygenases are implicated in the oxidative metabolism of a number of exogenous and endogenous substrates, the liver being the major site of CYP1A expression in fish.

The objective of this work was to analyze the sublethal effects of a commercial LAS mixture on larvae of the seabream using histopathological and immunochemical approaches.

Materials and methods

The assays were carried out following the procedures proposed by the OECD (1992), guideline 212 and the EPA (1996).

Fertilized seabream eggs were obtained from aquaculture facilities CUPIMAR S.A. (San Fernando, Spain) and CICEM El Toruño (El Puerto de Santa María, Cádiz, Spain) and maintained under controlled laboratory conditions until hatching, approximately 48 hours after fertilization. Commercial LAS, a mixture of C10-C13 homologues with all positional isomers except 1-phenyl (CAS Nº 68411-30-3) and an average molecular weight of 343 (sodium salt derivative), was supplied by PETRESA (Algeciras, Spain). After hatching, 25 neonate larvae, not older than 24 hours, were transferred into 1L glass test containers containing dissolved LAS at concentrations between 0.1 and 1.0 mg·L-1 (0.1; 0.2; 0.25; 0.3; 0.4; 0.5 and 1.0 $mg \cdot L^{-1}$) and exposed during 72 hours in a thermostatic chamber at constant temperature (22±1 °C) and photoperiod 12h light/12h darkness. Physico-chemical parameters, such as salinity, pH and dissolved oxygen were maintained at appropriate values similar to standard culture conditions (salinity 37±1; pH 8.1±0.1; %DO between 60-100%). The seawater used in the assays was obtained from a clean site in the Bay of Cádiz (Spain) in order to minimize the presence of LAS due to discharges of wastewater, and filtered through 0.45 mm glass fibre filters before use. LAS concentrations in seawater were below detection limit (<1$mg \cdot L^{-1}$). Exposure to the different concentrations was conducted in triplicates and three control assays were run simultaneously using pure filtered seawater.

Dead and living larvae were counted and recorded every 24 h. Dead individuals were removed from the test containers and the exposure solution was renewed completely each day in order to ensure constant concentrations. The mortality criterion used was the complete loss of heartbeat, which is generally combined with the loss of flotability and the appearance of a white coloration while the healthy larvae are normally transparent.

Those individuals which had survived until the end of the experiment (72 h) were fixed in 0.1M formaldehyde-phosphate buffer at pH 7.2 at least for 24 h before histopathological and immunohistochemical analysis. Part of the organisms was embedded in paraffine wax for immunohistochemical analysis. The rest was washed during 24 h in a saline solution containing saccharose 6.8%, adjusted to a pH of 7.4 with phosphate buffer and embedded in a commercial Historesin embedding kit (Leica Microsystems GmbH, Germany). In both cases, the preparations were cut into 6 µm-thick slices.

Histopathological analysis

Sections of Historesin embedded individuals were stained with haematoxylin-eosin and haematoxylin-V.O.F. (Gutierrez, 1967) for histomorphological observations under light microscopy (Leitz Diaplan, Germany).

Immunohistochemical CYP1A analysis

For immunohistochemical studies of CYP1A paraffin-embedded tissues were employed. Endogenous peroxidase activity was inhibited with methanol-H_2O_2 (6 ml of 3.3 % H_2O_2 in 10 ml methanol) for 5 minutes at room temperature. The sections were then transferred for 5 minutes to PBS with Triton X-100 (PBS-T), saturated in PBS-T with 0.5% casein for 30 min to block non-specific binding sites and incubated overnight at room temperature with a primary monoclonal rainbow trout CYP1A antibody (C10-7, Biosense AS, Bergen, Norway) diluted in PBS at 1:250. For further staining, the Vectastain ABC System (Vector Laboratories, Canada) including a biotinylated anti-mouse IgG secondary antibody was applied. To demonstrate the specificity of the CYP1A antibody, some sections were incubated with normal fish serum instead of the primary antibody.

Results

The type and degree of the observed effects of LAS exposure on seabream larvae appeared to depend on both surfactant concentration and exposure time. Observed mortality was similar to that in the control assays at exposure concentrations up to 0.3 $mg \cdot L^{-1}$. At 0.5 $mg \cdot L^{-1}$ about 50% of the exposed organisms survived until the end of the experiment (72 h), obtaining a LC_{50} (72 h) of 0.49 $mg \cdot L^{-1}$ (Hampel et al., 2000) . At the highest LAS concentration, survival was 0% after 48 hours of exposure.

Well developed larvae from control assays are straight organisms of between 2.5 and 3.0 mm length, with some pigmented areas in the body (Fig. 1). This morphology is maintained at lower LAS concentrations up to 0.25 $mg \cdot L^{-1}$, whereas at the highest sublethal exposure concentrations, surviving larvae presented frequently a characteristic curvature (Fig. 2). Comparing the yolk-sac from control larvae and those exposed to the highest sublethal concentrations, a peri-yolk sac edema can be clearly observed, combined with yolk retraction (Fig. 3a,b).

At histopathological level, control larvae showed the characteristic histological features described for this species on day 3 after hatching, with the yolk-sac

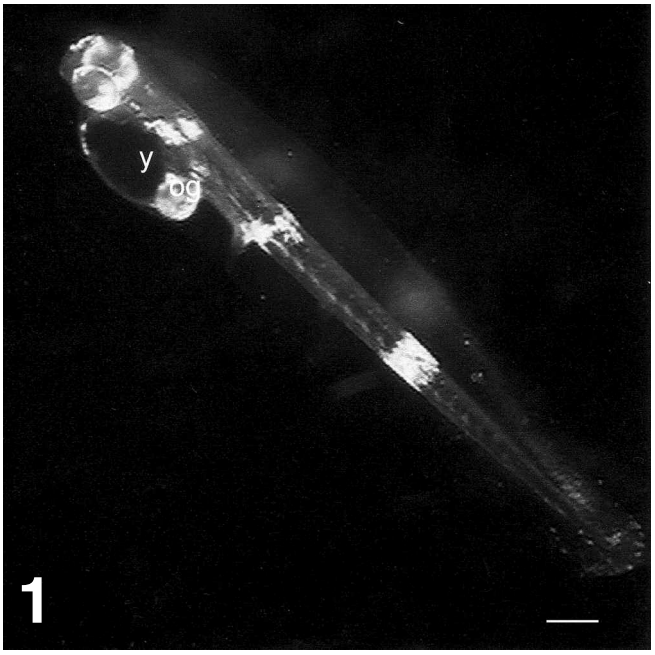

Fig. 1. Larva of *Sparus aurata* under no-contaminated conditions at two days after hatching. y: yolk, og: oil globule. Scale bar: 500 µm.

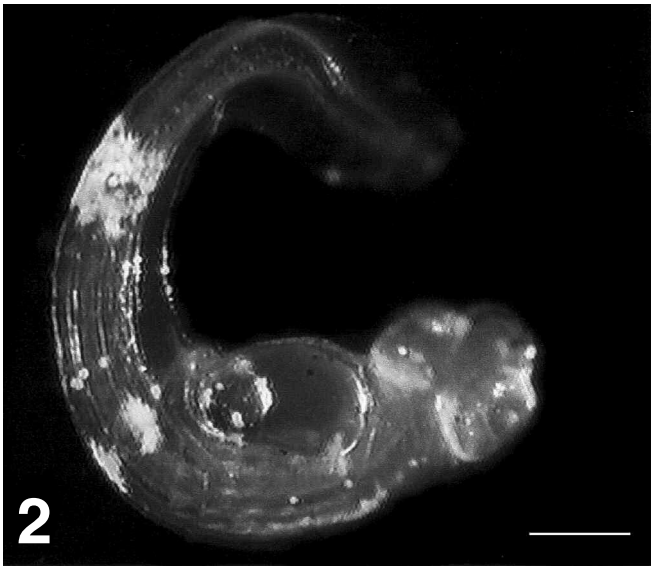

Fig. 2. Characteristic curvature of a seabream larva exposed to 0.5 $mg \cdot L^{-1}$ commercial LAS for two days. Scale bar: 500 µm.

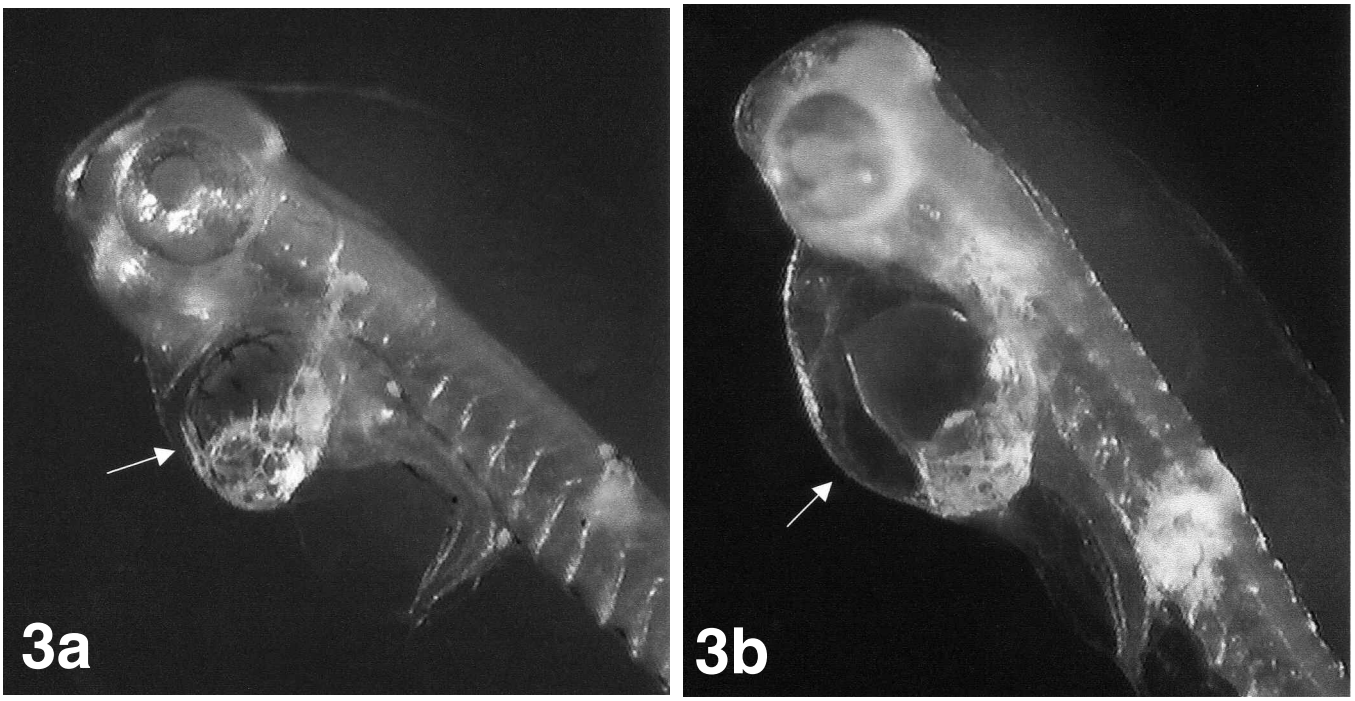

Fig. 3. Detail of the yolk sac of three-day-old sea-bream larvae under control conditions **(a)** and exposed to 0.5 $mg \cdot L^{-1}$ commercial LAS **(b)**. Under normal conditions, the yolk and the oil globule are closely envolved by the yolk sac epithelium **(a)** whereas at sublethal exposure concentrations **(b)**, severe cases of peri-yolk sac edema can be observed.

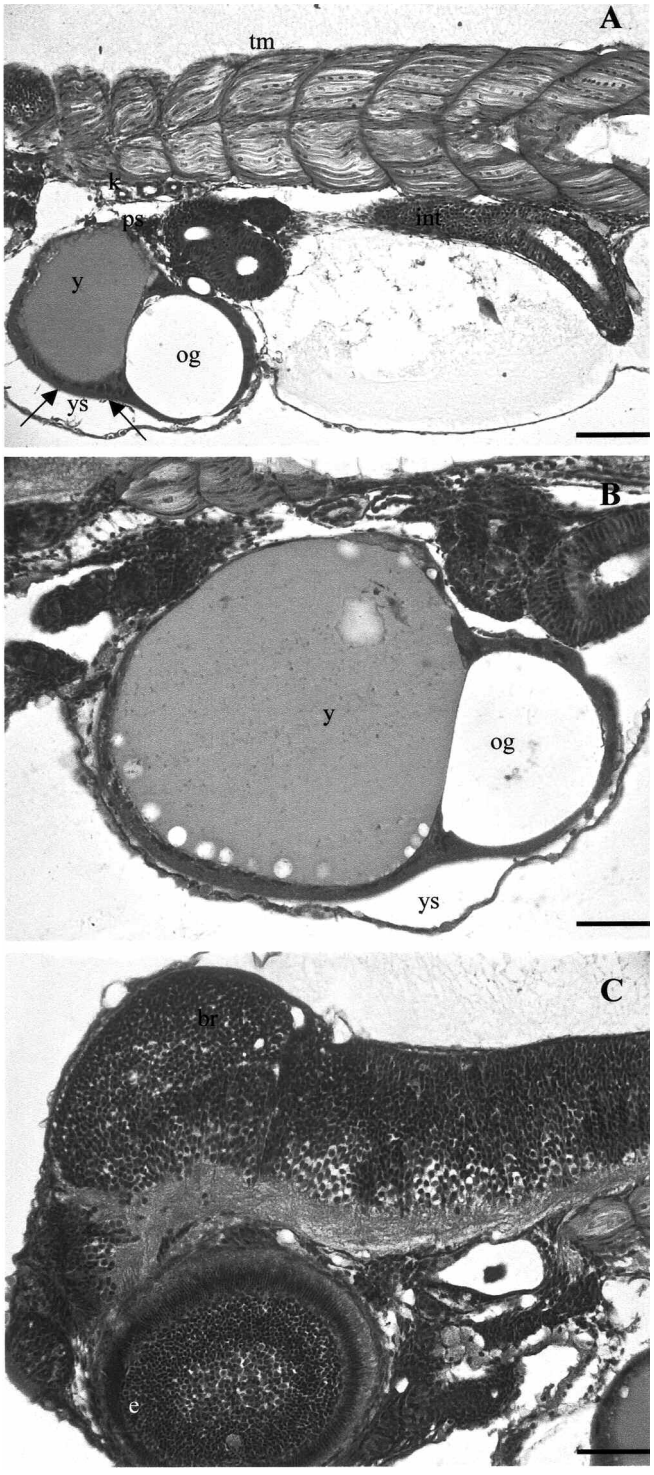

Fig. 4. Control specimens of *Sparus aurata* larvae, without any evident histopathological disorders. **A.** 3-DPH larva showing normal disposition of trunk musculature and the ventral disposition of a homogeneous acidophilic yolk sac surrounded by a monostratified layer of cuboidal cells (arrows). Scale bar: 125 µm. **B.** Detail of the yolk sac of a two-day-old sea-bream larva from a control assay. The yolk sac epithelium closely covers the yolk and the connected oil globule. The pericardial cavity presents normal development. Scale bar: 75 µm. **C.** Head of a 3 DPH control specimen showing normal disposition of the nervous tissue as well as the different layers of the retina. Scale bar: 75 µm. ys: yolk sac; y: yolk; og: oil globule; int: intestine; tm: trunk musculature; br: brain; e: eye; k: kidney; ps: pancreatic structure.

content almost absorbed (Fig. 4a-c). At the lowest LAS exposure concentration (0.2 mg·L^{-1}), digestive tract (Fig. 5a) and nervous system (Fig. 5a, b) do not show apparent alterations. Nevertheless, first cases of peri-yolk sac edema can be observed occasionally, increasing the space between the trophoblastic sincitium and the epidermis (Fig. 5b,c), as well as a certain retraction and deformation of the yolk-sac. The pericardial cavity does not show any alteration (Fig. 5c).

At an exposure concentration of 0.25 mg LAS·$L^{-1,}$ first signs of disorganisation of the brain cells (Fig. 6a) can be observed and peri-yolk sac edema appear more frequently, combined with a strong deformation of the yolk-sac and alterations in the oil globule (Fig. 6b, e). Eventually, the oil globule appears separated from the yolk-sac, without any contact with the trophoblastic sincitium (Fig. 6c,e). At this exposure concentration, the pericardial cavity frequently seems to increase indicating pericardial edema. Furthermore, hepatic tissues indicate first signs of necrosis (Fig. 6b). The skeletal musculature shows almost in all studied individuals serious degradation (which could explain curvature of exposed organisms), appearing the muscle fibres torn transversally and longitudinally (Fig. 6d).

At the highest sublethal exposure concentration (0.5 mg·L^{-1}), almost all surviving organisms showed severe curvature of the body, a general sign of toxicity (Fig. 7a). This phenomenon was also observed at lower LAS concentrations, increasing in frequency with exposure concentration. The developing hepatic structures show cytoplasmatic vacuolisation (Fig. 7b), pericardial edema (Fig. 7c,d) and disorganisation of trunk musculature (Fig. 8a) with important necrosis and vacuolisation (Fig. 8c) in some cases. Additionally, severe necrosis and general disorganisation in the nervous system of the brain region (Fig. 7d) and severe necrosis (Fig. 9b) was observed in the highest concentration.

At this exposure concentration (0,5 mg LAS·L^{-1}), the trophoblastic sincitium, normally composed by a simple cell layer, increased considerably in thickness with the basal nuclei disposed irregularly. At the same time, important deformations of the yolk-sac and the lipid globule were observed (Fig. 8a, b). The intestine epithelium, which in control organisms is formed by a straight unfolded tube composed of a simple cell layer, presents dense internal folds and cell proliferations in the hindgut region (Fig. 9b) which seem to obstruct the digestive tube and may hinder excretion of necessary enzymes and the regular passage of alimentary particles.

Immunohistochemical analysis of CYP1A

CYP1a inmunohistochemical staining of seabream control larvae was previously studied, showing positive immunostaining in organs of the digestive tract, both the liver and the alimentary canal, whereas no immunoreactivity was seen in the exocrine pancreas, as observed in control larvae of the present study. In these LAS-exposed larvae only a weak increase of CYP1A

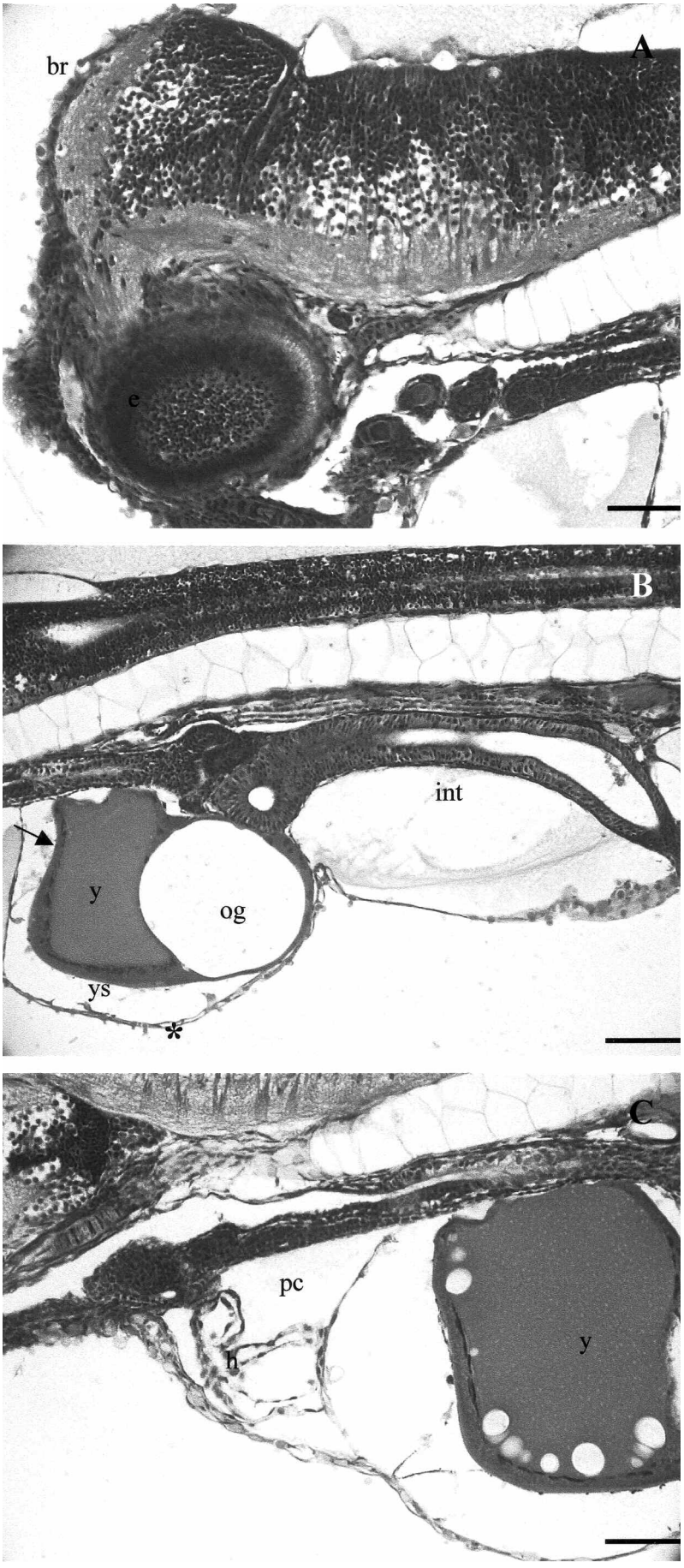

Fig. 5. Larvae of *Sparus aurata* at the lowest LAS exposure concentration (0.2 mg·L^{-1}). Neither the nervous system, nor the digestive tract show apparent alterations **(A and B)** First cases of peri-yolk sac edema, showing an increase in the space between the trophoblastic syncitium and the epidermis (asterisk), as well as a certain retraction and deformation of the yolk-sac (arrow). The pericardial cavity does not show any alteration **(C)**. ys: yolk sac; y: yolk; og: oil globule; int: intestine; pc: pericardial cavity; br: brain; e: eye. Scale bars: AA, C, 75 µm; B, 125 µm.

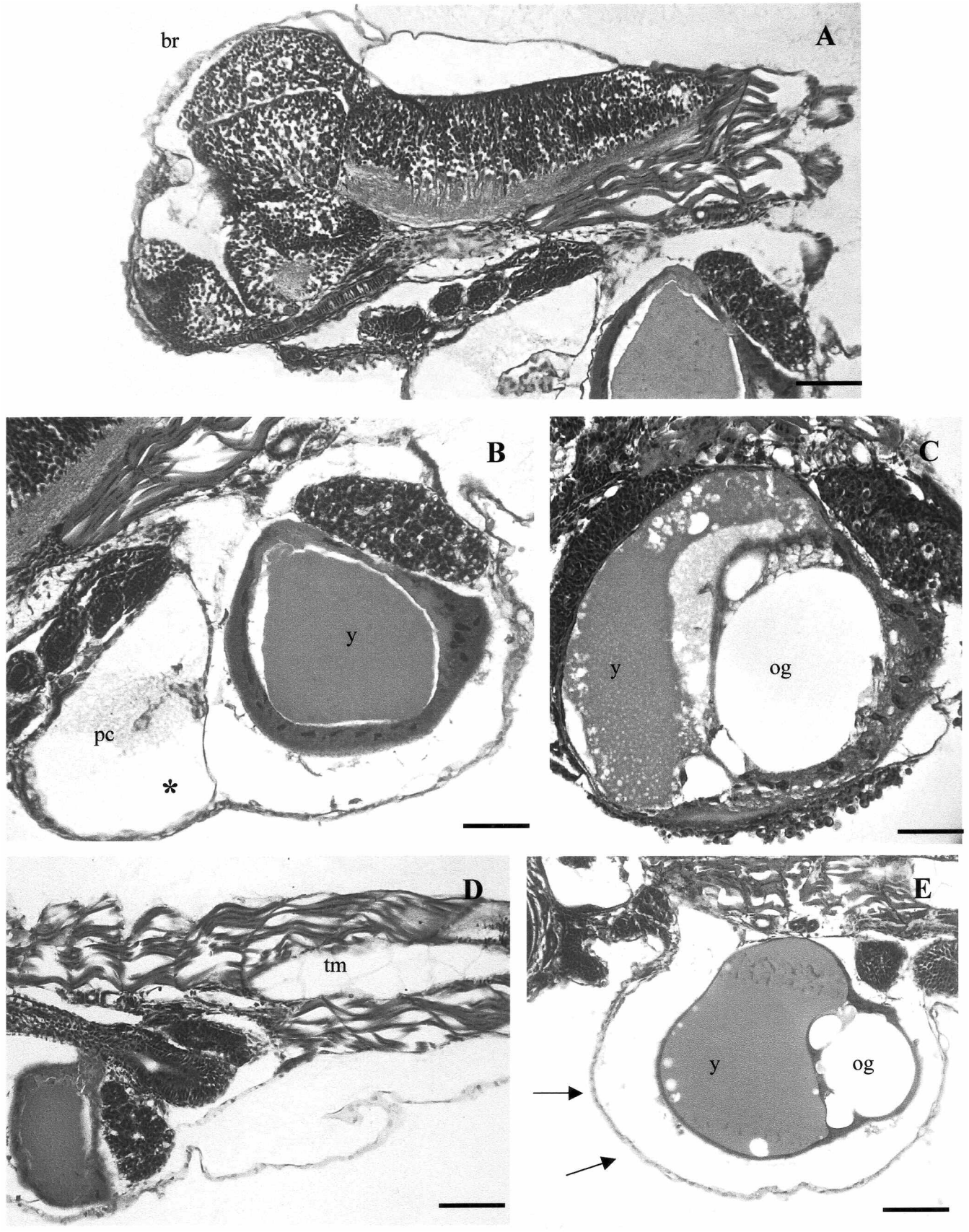

Fig. 6. Larvae of *Sparus aurata* exposed to 0.25 mg.L^{-1} of commercial LAS showing the nervous system without any apparent disorders **(A)**. Histopathological disorders like pericardial edema **(B)** (asterisk), displacement of the oil globule from the periphery to the centre of the yolk sac **(C)**, muscular disorganization **(D)** and yolk sac edema **(E)** are frequent in exposed fish. ys: yolk sac; y: yolk; og: oil globule; pc: pericardial cavity; br: brain; tm: trunk musculature. Sccale baars: A, D, 125 µm; B, E, 75 µm; C, 50 µm.

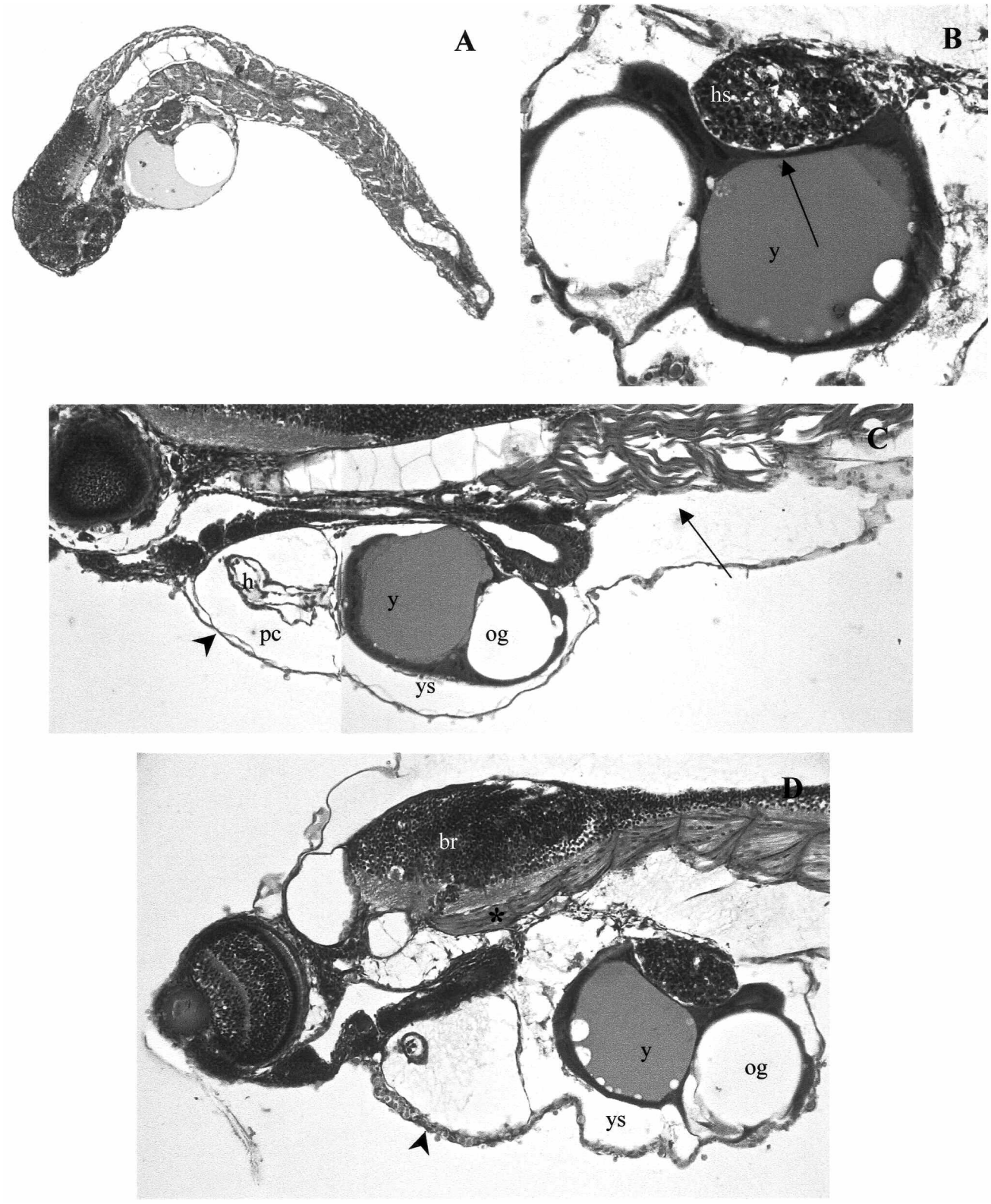

Fig. 7: Larvae of *Sparus aurata* exposed to 0.5 mg.L^{-1} of commercial LAS. A characteristic curvature is seen in seabream larvae exposed to commercial LAS **(A)**. Moreover, the developing hepatic structures show cytoplasmatic vacuolisation **(B)**. Disorganization of trunk musculature (arrows), pericardial edema (arrowhead), as well as necrosis and disorganization of the nervous system (asterisks) are also observed **(C and D)**. ys: yolk sac; og: oil globule. A, 175 µm; B, 50 µm; C, D, 125 µm.

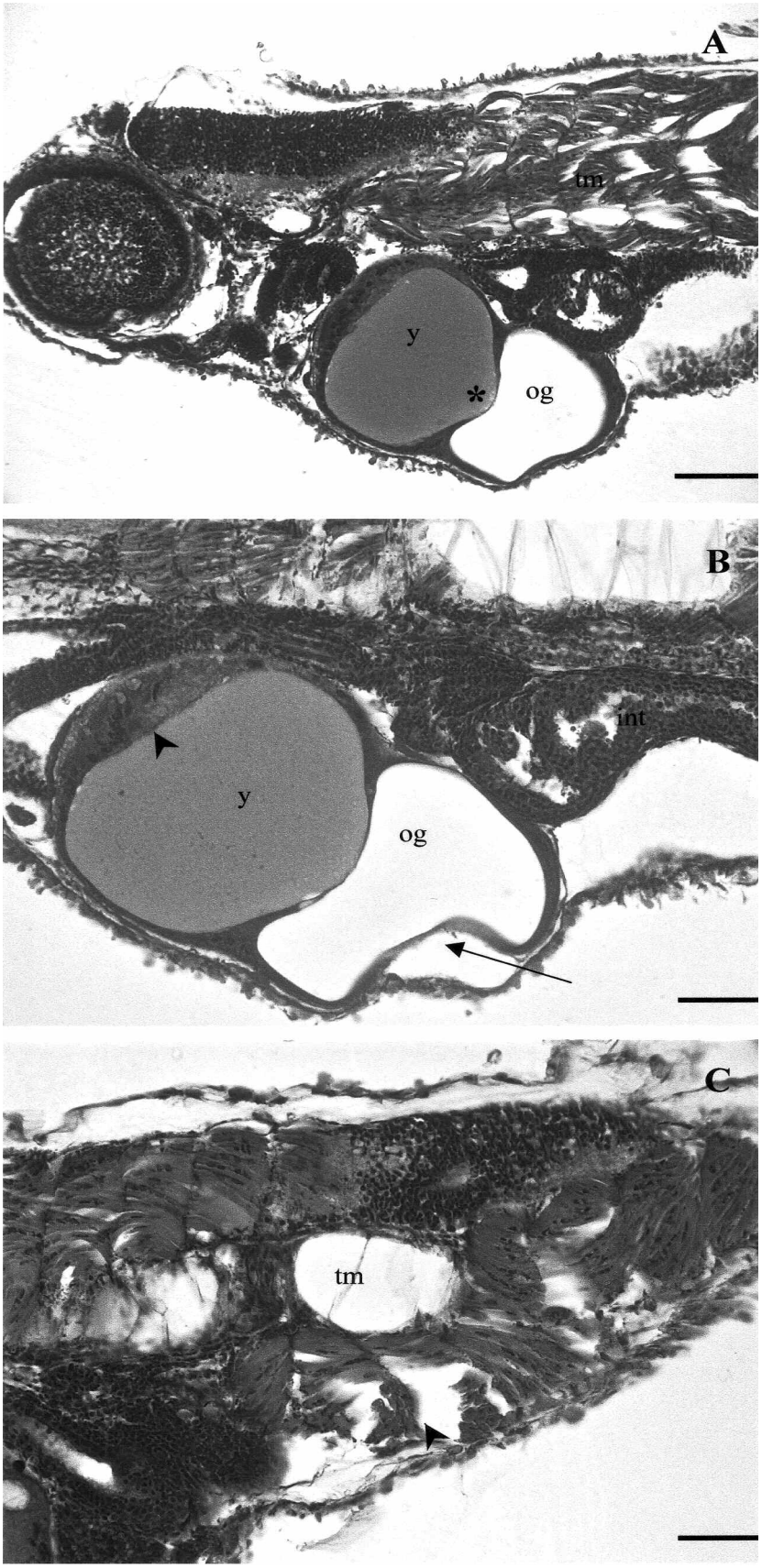

Fig. 8. Larvae of *Sparus aurata* exposed to 0.5 mg.L^{-1} of commercial LAS, showing a shrinkage of the oil globule (arrow) and deformation of the yolk sac (asterisk) **(A and B)** as well as a certain disorganization of the cell layers from the retina **(A**). The trophoblastic syncitium increases in thickness (arrowhead) **(B)**. Skeletal musculature is severely damaged, presenting important necrosis and vacuolisation **(C)** (arrowhead). ys: yolk sac; og: oil globule; tm: trunk musculature. Scale bars: A, 125 µm; B, C, 50 µm.

was observed in the hepatic vascular system at LAS concentrations ranging between 0.4 and 0.5 mg·L^{-1}. No clear tendency of CYP1A response in relation to different LAS concentrations could be established (Fig. 10a-c).

Discussion

Even if there is a considerable number of publications about the effects of surfactants on aquatic vertebrates and invertebrates (Holman and Macek, 1980), including valuable works at histopathological level in adults (Verma and Mohan 1976; Hofer et al., 1995), information about marine organisms is scarce and no studies about internal changes in early-life stages of marine fish due to exposure to surfactants have been reported. Nevertheless, a similar experiment was carried out by Henry et al. (1997) who exposed newly hatched zebrafish larvae (*Danio rerio*) to increasing concentrations of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). These authors detected a relationship between reductions in blood flow and the development of pericardial and yolk-sac edema, and observed, coincident with the onset of pericardial edema, a slowed blood flow in the trunk of TCDD-treated zebrafish larvae. Additionally, endomysial edema of the skeletal muscle occurred after the onset of peripheral circulatory dysfunction. In our work, heart beat rate was not studied, but a decrease of heart beat frequency of almost dead animals was always observed as it was one of the employed mortality criteria. As consequence of a less effective blood pumping in affected larvae, normal blood supply to the different organs is not guaranteed which may provoke several types of lesions and dysfunctions.

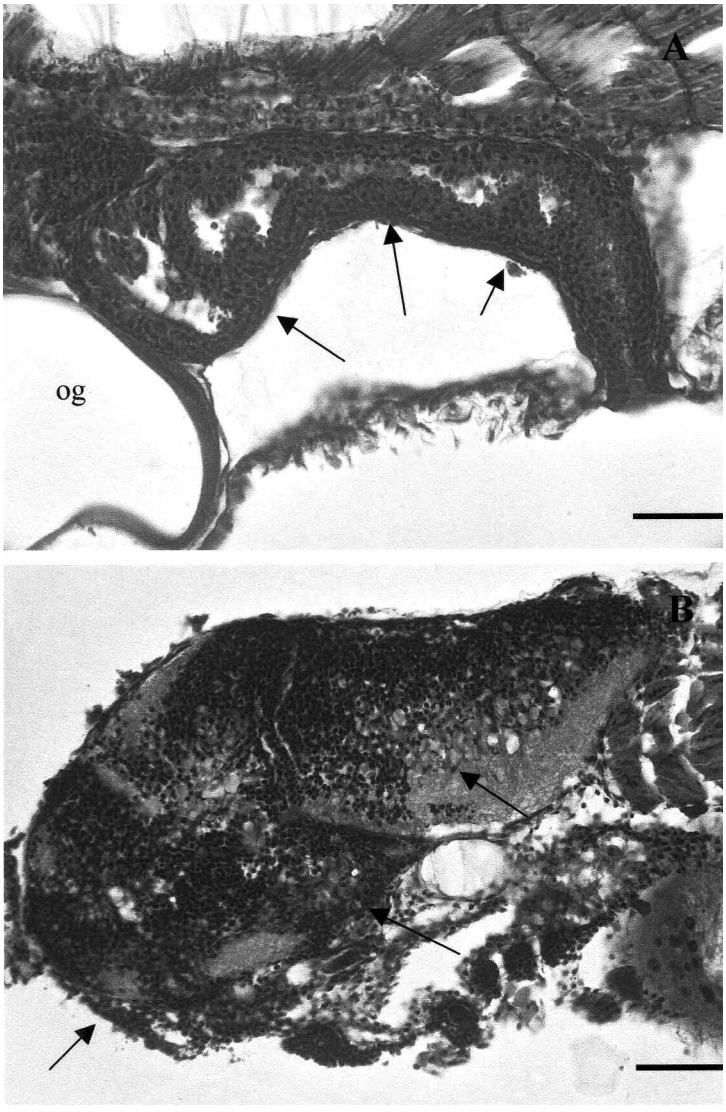

Fig. 9. Larvae of *Sparus aurata* exposed to 0.5 mg.L^{-1} of commercial LAS, showing severe curvature of the body **(A)**. The intestine epithelium presents dense internal folds and cell proliferations in the hindgut region with a severe occlusion of the digestive lumen (arrows) **(B)**. The nervous tissue is severely disorganized (arrows) **(C)**. Scale bar: 75 µm.

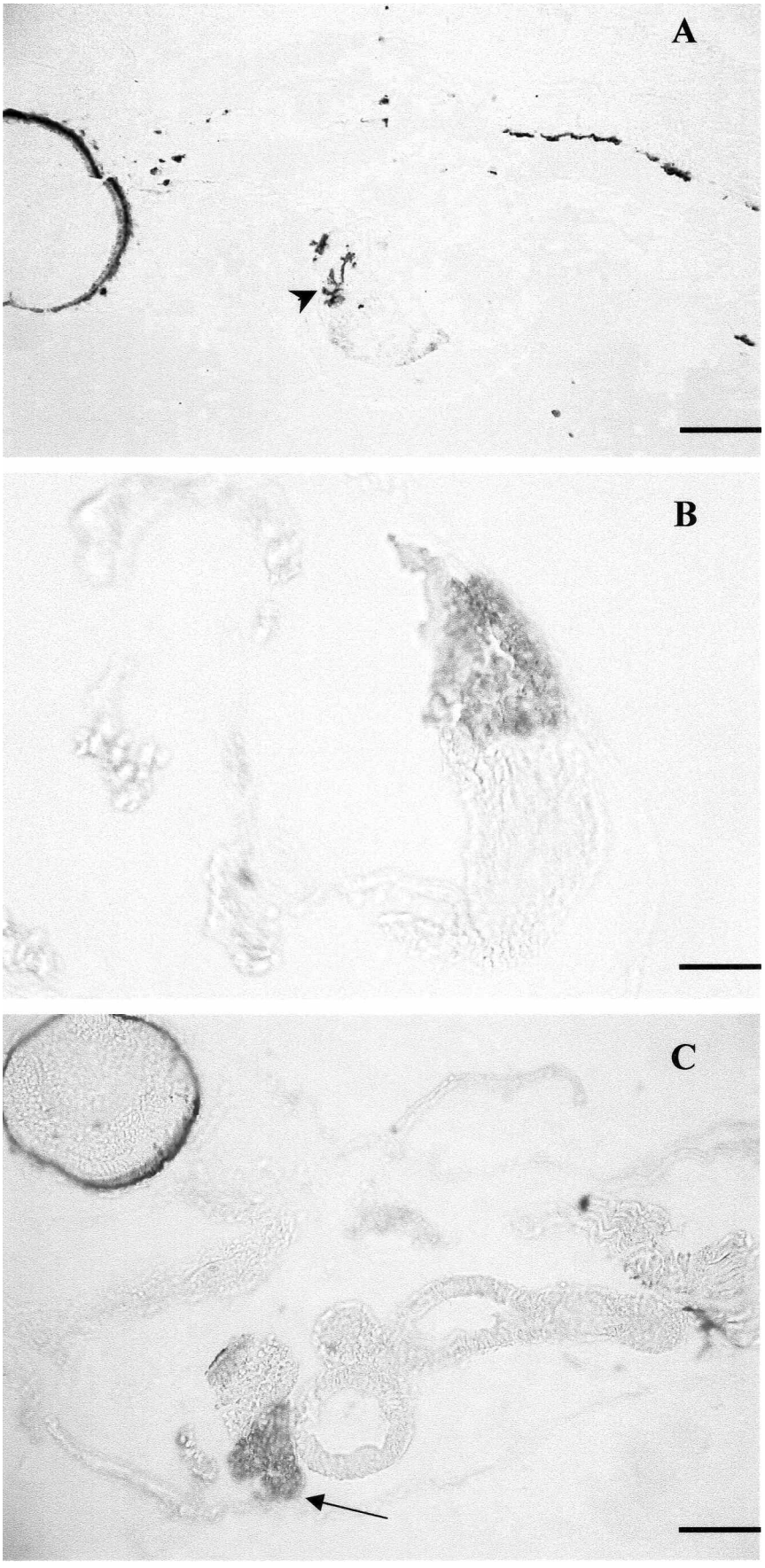

Fig. 10. Immunohistochemical distribution of CYP1A in control and exposed *Sparus aurata* larvae. **A.** Larvae of *Sparus aurata* control specimens showing CYP1A immunostaining mainly located in vascular structures of liver (arrowhead). **B and C.** CYP1A immunoreactivity in hepatocytes of the liver at LAS concentration 0.3 mg·L^{-1} (arrows). Scale bars: A, C, 125 µm; B, 75 µm.

The appearance of curved larvae at higher sublethal exposure concentrations, for example, could also be related to reduced blood flow supplying the trunk skeletal muscle. In histological samples, affected larvae showed altered muscle structure composed by muscle fibres which seem to be ripped up. Abnormal development of the spinal column, as well as abnormal head and eye development are one of the most conspicuous damages in early-life-stages and it has to be pointed out that morphological aberrations are not particularly pollutant-specific and may also be caused by natural stressors (von Westernhagen, 1970). Affected individuals may not be able to perform correct movements affecting both, feeding and/or predation and escape which represents a clear disadvantage over not affected animals.

Histopathological analysis

In the seabream, hatching occurs approximately 48 hours after fertilization, depending in a certain degree on environmental conditions such as temperature and photoperiod. The alimentary channel of the newly hatched organisms is a more or less straight tube, closed at both ends and constituted by a simple columnar or pyramidal epithelium that lies on a layer of spindle-shaped cells (Sarasquete et al., 1993; Calzada et al. 1998). At this moment, the larvae are equipped with a big yolk-sac and float inactively at the water surface. At the third day after hatching (72 h) the larvae begin to open their mouth in order to start to feed with the first exogenous alimentary particles. This is a critical moment in the life of each teleost, due to the fact that the intestine has to undergo several functional and morphological modifications for the larvae to support these changes in the diet and to absorb and digest effectively exogenous food. All alterations in the complete digestive system of the larvae in this transformation from endogenous to exogenous feeding produce additional stress which could reduce its already debilitated resistance. Particularly marine cultured species as the sea-braem present high larval mortality rates during this period (Fyhn, 1989). At this development stage (3 DPH), the intestine can be divided into three different zones depending on the constituting cells: the midgut, which represents the longest segment of the gut forming a loop close to the yolk-sac, the hindgut, which bends down from the posterior curvature of the gut, and the rectum, represented by the short caudal segment which communicates with the exterior via the anus (Calzada et al., 1998). At hatching, the gut is composed by quite undifferentiated cells but presents shortly before first exogenous feeding three clearly separated regions, prepared to digest and absorb exogenous food. At highest exposure concentrations (0.5 $mg \cdot L^{-1}$) severe obstruction of the hindgut can be observed, probably due to proliferation of the intestinal cells which also present changed morphology and disorganisation. Additionally, the hindgut which in a normally developed organism is constituted by a straight tube presented several dense pleats and folds. Functionality of this altered intestine will be probably reduced, by obstruction or altered digestive activity, presenting an additional difficulty to the already critical phase of the onset of exogenous feeding inhibiting absorption and transformation of alimentary particles into profitable energy.

The absorption and digestion of exogenous food particles includes the existence of an exocrine pancreas responsible for the synthesis and secretion of digestive enzymes, even if no gastric enzymes cannot be synthesised yet due to the absence of a functional stomach. At the age of 3 days, seabream larvae have already started to develop hepatic and pancreatic structures presenting normally developed hepatocytes (Sarasquete et al., 1993). Necrosis or ballooning degeneration at higher sublethal exposure concentrations ($0.5\ mg \cdot L^{-1}$) could lead to altered physiological functioning.

Only by comparison of enlarged binocular photographs of the details of the yolk-sac of a control and an exposed organism, an increase of the yolk-sac of the affected organisms can be perceived. In addition to external observations of peri-yolk sac edema in exposed individuals, at histopathologica level severe deformations of the yolk-sac as well as displacement of the lipid globule indicated that these structures were affected by LAS exposure in relation to its exposure concentration. It is possible that, apart from reduced blood flow through this organ, the lipophilic nature of the surfactant alters its composition and, as consequence, its general appearance. Reduction of available nutrient reserves could lead to sooner exhaustion of the yolk and could make premature exogenous feeding necessary without sufficient preparation of the digestive region or produce starvation if the mouth has not been opened yet. During the time of endogenous feeding, nutrient absorption is produced by the sincitium surrounding the yolk-sac without the intestine cells taking part. In some cases, where the lipid globule is displaced towards the interior of the yolk, no contact with the trophoblastic sincitium is established and the lipid reserves are not susceptible to be used. This must produce an alteration in the nutrient proportions which are supplied to the larvae occurring possible deficits in the lipid supply.

In combination with these internal changes, pigmentation of the eyes initiates as the ability of larval fish to feed based on optic stimuli depends on the development of the optical elements of the eye, as well as the synaptic connections between the neurones of the retina and their connections in visual brain centres. Histological investigations of many species have revealed the presence of a differentiated retina containing photoreceptors and neural connections to the optic tectum by the time of feeding (Powers and Raymond, 1990). In the preparations of the present study, three different cell lavers can be clearly observed in eyes of the control organisms, surrounding a mass of

Table 1. Measured LAS concentrations in different estuarine and coastal environments (León, 2001).

SITE	LAS CONCENTRATION [$\mu g \cdot L^{-1}$]	REFERENCE
Estuarine waters		
Barbate (Spain)	6.1-9.8	González-Mazo et al., 1997
Scheldt (Belgium)	<0.5-9.4	Matthijs and Stalmans, 1993
Venice Lagoon (Italy)	1.3-256	Marcomini et al., 2000
Krka (Croatia)	0.9-391	Terzic and Ahel, 1994
Tamagawa (Japan)	8.1-444	Takada and Ogura, 1992
Coastal waters		
Bay of Cádiz (Spain)	3.5-51.2	González-Mazo et al., 1997
Caño de Sancti Petri (Spain)	2.7-1687	González-Mazo et al., 1998
Venice Lagoon (Italy)	1.2-296.5	Stalmans et al., 1990
Venice Lagoon (Italy)	1.4-2.4	Marcomini et al., 2000
North Sea	<0.4-1.2	Matthijs and Stalmans, 1993
Golf of Thermaikos (Greece)	10-60	Kilikidis et al., 1994
Bay of Tokio (Japan)	<3-14	Hon-nami and Hanya, 1980

homogeneously distributed circular cells with a clearer part in its centre where the cell density is lower. This defined organisation is observed also in exposed organisms until LAS concentrations up to 0.4 $mg \cdot L^{-1}$, whereas at the highest exposure concentration (0.5 $mg \cdot L^{-1}$), a certain disorganisation of the surrounding cells was detected which could have some influence on the vision quality. Failure to capture preys, which can be caused by insufficient vision, leads to rapid starvation and death and the larval stages of numerous species suffer from high mortality (Hunter, 1981). Actually, the success of feeding by visual detection of food particles depends on both, correct eye as well as neuronal development. Alteration of brain cells, which was observed at the highest sublethal exposure concentrations, can cause additionally other adverse effects such as failure in movement coordination leading finally to less effective behaviour patterns.

Immunohistochemical analysis of CYP1A

Cytochrome P450s monooxigenases (CYP1A) have proved to be indicators of several endogenous substrates and organic lipophilic compounds (Sarasquete and Segner, 2000; Ortiz-Delgado and Sarasquete, 2003), but no studies have been performed with the anionic surfactant Linear Alkylbenzene Sulphonate (LAS), yet. Surface active compounds as surfactants are composed of a hydrophilic part and a lipophilic alkyl chain. The higher the number of carbon atoms in the alkyl-chain, the more lipophilic is the molecule (Holman and Macek, 1980). It is possible that due to the presence of a polar group, LAS may not be an adequate CYP1A inductor, and further studies should be carried out to find out whether different homologues present different responses depending on the length of the alkyl chain.

Generally, potentially hazardous concentrations of LAS do not exist in open waters where dilution, dispersion and biodegradation are important factors in the reduction of exposure concentrations near discharge points. LAS concentrations have been analysed in estuarine and coastal waters by several authors showing wide concentration ranges in these environments (Table 1). Several of the cited sites represent potentially harmful concentrations for existing seabream populations as they are higher than 0.25 $mg \cdot L^{-1}$, the lowest concentration where sublethal changes have been detected in this study.

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