Histol Histopathol (1995) 10: 781-787

Histology and Histopathology

Morphological and histochemical changes in the liver and pancreas of gilthead, *Sparus auratus* L., induced by acute action of the anionic detergent, sodium dodecyl sulphate

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Summary. This paper reports the morphological and histochemical changes in the livers and pancreas of giltheads (*Sparus auratus*, L.) induced by acute action of the anionic detergent, sodium dodecyl sulphate (SDS). Sixty-five giltheads were exposed to SDS concentrations of 5, 8.5, 10 and 15 mg/l. The surface tension induced at each concentration was determined and the LC50 calculated. Morphological changes dependent on detergent concentrations and length of exposure were seen. Histochemical techniques showed alterations in carbohydrates and proteins, which may interfere with liver and pancreas function.

Key words: Liver, Pancreas, Detergent, Sodium dodecyl sulphate, *Sparus auratus*, Gilthead

Introduction

Detergents pollute the environment and their toxic effects can be demonstrated. SDS is one of the most widely commercialized domestic detergents in the USA, Japan and Western Europe; more than 70 million Kg are sold per year. Primary bio-degradation of SDS is rapid, disappearing in less than a day in cultures or in river water test (Swisher, 1970). However, although it loses its original identity, the inconveniences and the effects of the intermediate breakdown products persist until bio-degradation is complete (Kimerle and Swisher, 1977; Oba et al., 1977; Bukema et al., 1982).

The mechanisms by which detergents produce their effects are not well understood (Helenius and Simons, 1975). It is thought that the fall in surface tension they induce is the main cause of death (Prat and Giraud, 1964; Bock, 1965; Mann, 1972). Surface tension values in this experiment reached as low as 49.9 mN/m. Even lower values have been measured in the Bay of Cádiz (Flores et al., 1979). It is therefore important to determine the cause of death and knowledge in this area would be valuable since giltheads are exposed to lethal doses.

Independent from the mechanism that causes death, it is of interest to understand the histomorphological changes that occur and, through histochemical methods, to appreciate alterations induced in carbohydrates and proteins. Gilthead tissues absorb this detergent, and its high affinity for lipid membranes provokes changes in the phospholipid bi-layer. The behaviour of certain bio-macromolecules can help explain the effects of detergent on the tissue.

This investigation shows some morphological and histochemical changes produced in *Sparus auratus*, L. by the action of a common detergent, sodium dodecyl sulphate (SDS), and their effect on survival. Research on the relationship of SDS with this species is particularly appropriate because of its importance in the fishing industry and in pisciculture (Arias et al., 1976; Varona, 1993).

Materials and methods

The fish used in this investigation were sixty-five juvenile giltheads, six months old, between 12 and 14 cm in length and weighing from 30 to 40 g. All were born and raised on a fish farm. Five specimens were used as controls and the remainder divided into four groups, A, B, C and D, which were exposed to concentrations of 15, 10, 8.5 and 5 mg/l of SDS (Merck, Spain), respectively, until 50% of the animals in each treatment died. The LC50 causing death in a period of 96 hours was determined according to Sprague (1976) and Loomis (1982).

Each group was maintained in a PVC tank containing 200 litres of sea water, whose characteristics were: salinity $30^0/00$, pH 7.4, temperature 16-18 °C,

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surface tension 72.7 mN/m, dissolved oxygen 8-8.6 mg/l, absent of heavy metals, and contamination due to aerobe and anaerobe microorganisms. To avoid variations in detergent concentration, test solutions were changed every 12 hours. The bio-degradation occurring in this time is less than 10% of the initial concentration (Flores et al., 1980).

Surface tension was measured using a Lauda TE 1 C/2 with SAE+KM3 tensiometer. An initial reading was taken for all groups. Group B was further measured at 6 h and groups C and D at 12 h.

After the animals died from exposure to the detergent, their liver-pancreas were removed. Samples were fixed in 10% v/v formol buffered with phosphate buffer 0.1M, pH 7.2, dehydrated in increasing concentrations of alcohol, cleared with benzol and embedded in semisynthetic paraffin wax with a mean fusion point of 54-56 ºC. Sections were cut at 5 µm.

Harris's haematoxylin and acetic eosin, Harris's haematoxylin-VOF (Gutiérrez, 1967) and Gridley's reticulum stain (Gridley, 1951) were employed as general stains. The histochemical techniques used are shown in Table 1.

Table 1. Histochemical reactions.

REACTIONS	FUNCTIONS AND/OR COMPOUND SHOWN
Schiff reagent (Pearse, 1960)	Free aldehydes
Periodic acid-Schiff (PAS) (McManus, 1948)	Glycogen, neutral mucosubstances and/or glycoproteins, sialic acid
Diastase-PAS (Lillie and Greco, 1947)	Neutral mucosubstances and/or glycoproteins, excepting glycogen
Alpha amylase-PAS (Lillie and Greco, 1947)	Neutral mucosubstances and/or glycoproteins, excepting glycogen
Chlorhydric hydrolisis-PAS (Martoja and Martoja-Pierson, 1970)	Neutral mucosubstances and/or glycoproteins, excepting sialic acid
Colloidal iron-potassium ferricyanide (Hale, 1957)	Acid mucopolysaccharides
Alcian blue pH 2.5 (Martoja and Martoja-Pierson, 1970)	Acid glycoconjugates (carboxylated and sulphated)
Hyaluronidase-alcian blue pH 2.5 (Pearse, 1960)	Acid glycoconjugates, excepting hyaluronic acid
Alcian blue pH 1 (Martoja and Martoja-Pierson, 1970)	Sulphated glycoconjugates
Alcian blue pH 0.4 (Martoja and Martoja-Pierson, 1970)	Very sulphated glycoconjugates
Toluidine blue (Martoja and Martoja-Pierson, 1970)	Metachromasia, acid mucosubstances
Bromophenol blue-Hg (Chapman, 1971)	Proteins in general
Hartig Zacharias method (Martoja and Martoja-Pierson, 1970)	Siderophile proteins
Nihydrin-Schiff (Pearse, 1960)	Proteins rich in -NH2 groups (lysine)
1-2 Naphtoquinone-4-sulphonic acid, sodium salt (NQS) (Lillie et al., 1971)	Proteins rich in arginine
p-Dimethylaminobenzaldehyde (Barka and Anderson, 1963)	Proteins rich in tryptophan
Ferric ferricyanide-Fe (III) (Chevremont and Frederic, 1943)	Proteins rich in SH groups (cysteine) and other reductor groups
Thioglicolate-potassium ferricyanide-Fe (III) (Chevremont and Frederic, 1943)	Proteins rich in S-S groups (cystine)

Results

The LC50, extrapolating the results on semi-logarithmic paper and noting the time when 50% of fish in each group died, at 96 h for *Sparus auratus*, L. was 6.1 mg/l. Table 2 shows time of death and the resultant surface tension at each concentration.

Table 3 shows the histochemical behaviour of proteins and carbohydrates.

Group control. (Figs. 1a, 2a, 3a, 4a and 5a).

Liver and pancreas formed a single topographical unit. The pancreas penetrated the liver, following and surrounding the branches of the portal vein and extending throughout as cellular islets (intrahepatic pancreas).

The hepatocytes were cuboidal in form with well-defined limits and a central nucleus. They were arranged in radial strings around a central vein. Within these hepatocyte strings the biliary canaliculi could be seen. In hepatocyte cytoplasm there was a large amount of glycogen, a moderate quantity of proteins in general and a weak presence of siderophilic proteins.

Cells of the exocrine pancreas showed prismatic morphology and a central nucleus. Their cytoplasm was dense and contained much rough endoplasmic reticulum. Many zymogen granules could be seen at the apical extremity. In the cytoplasm of pancreatic cells siderophilic proteins and proteins in general were represented strongly. There were no neutral or acidic mucosubstances.

Group A

At 15 mg/l part of the radial arrangement of hepatocytes was lost. The majority of pancreatic cells contained no zymogen and those that did had very little.

Table 2. Time to death of 50% of fish and the surface tension resulting at each SDS concentration.

SDS CONCENTRATION	TIME TO DEATH	SURFACE TENSION
15 mg/l	30 min	49.9 mN/m
10 mg/l	6 hours	53.3 mN/m
8.5 mg/l	12 hours	54.3 mN/m
5 mg/l	250 hours	56.0mN/m

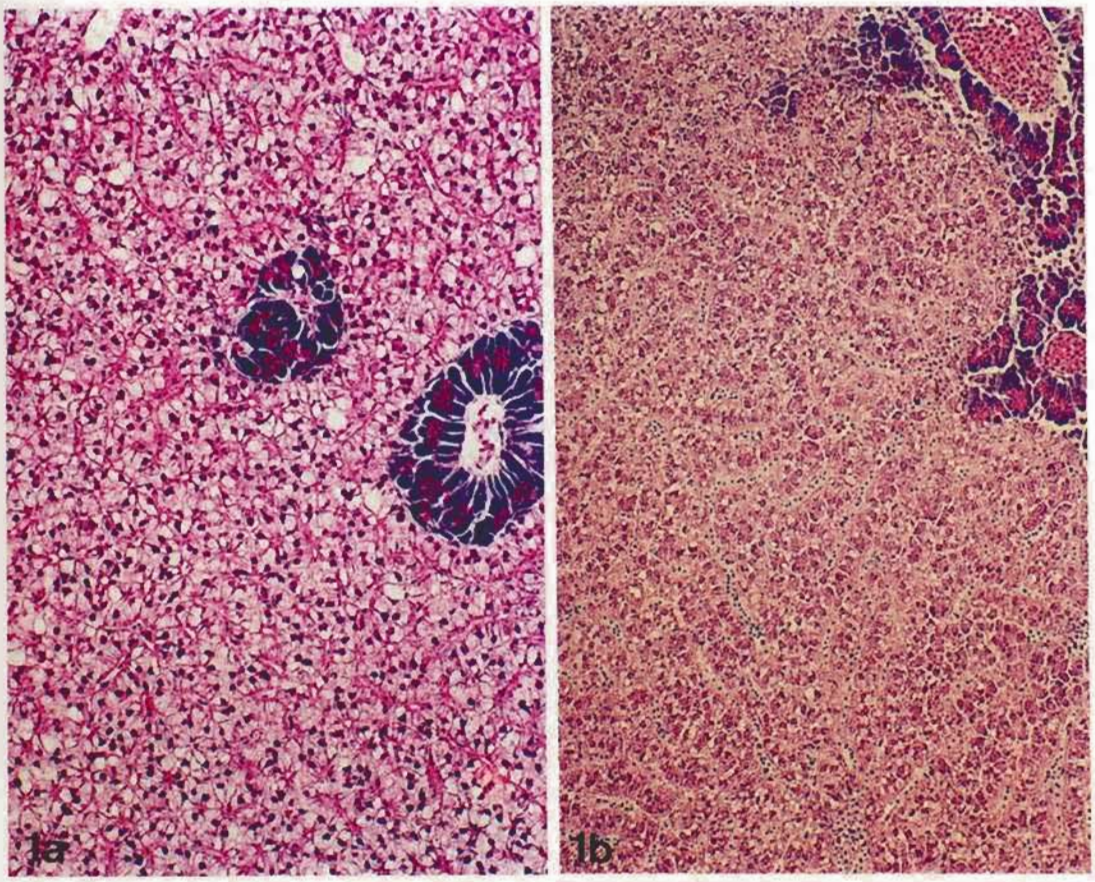

Fig. 1. Liver pancreas of *Sparus auratus*, L. stained with haematoxylin-eosin. **a.** Control. **b.** Group C. x 250

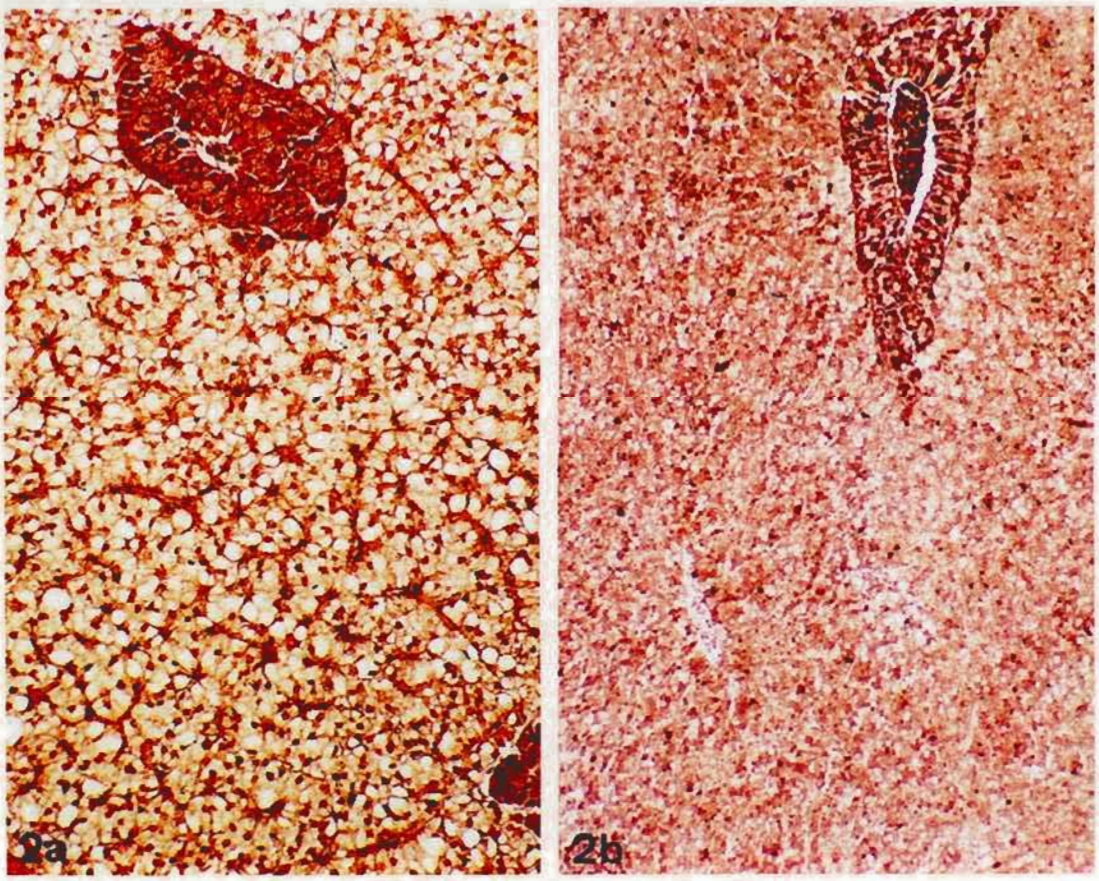

Fig. 2. Liver pancreas of *Sparus auratus*, L. stained with Gridley's reticulum stain. **a.** Control, **b.** Group D. x 250

Table 3. Histochemical reactions of carbohydrates in the liver-pancreas of *Sparus auratus*, L. against different concentrations of SDS.

REACTION	LIVER PARENCHYMA					PANCREATIC CELLS				
	Ctrl.	15	10	8.5	5	Ctrl.	15	10	8.5	5
Schiff	0	0	0	0	0	0	0	0	0	0
PAS	3	1	3*	0	0	0	0	0	0	0
Diastase-PAS	1	0	0	NT	NT	NT	NT	NT	NT	NT
Alpha amilase-PAS	0	0	0	NT	NT	NT	NT	NT	NT	NT
Chlorhydric hydrolisis-PAS	0	0	0	0	0	0	0	0	0	0
Colloidal iron-potassium ferricyanide	0	0	0	0	0	0	0	0	0	0
Alcian blue pH 2.5	0	0	0	0	0	0	0	0	0	0
Hyaluronidase-alcian blue pH 2.5	0	0	0	0	0	0	0	0	0	0
Alcian blue pH 1	0	0	0	0	0	0	0	0	0	0
Alcian blue pH 0.4	0	0	0	0	0	0	0	0	0	0
Toluidine blue (metachromasia)	Ort	Ort	Ort	Ort	Ort	Ort	Ort	Ort	Ort	Ort
Bromophenol blue-Hg	2	1	1	1	2	3	1	1	1	2
Hartig Zacharias method	1	1	1	1	1	3	1	2	2	2
Ninhydrin-Schiff	0	0	0	0	0	0	0	0	0	0
NQS	0	0	0	0	0	0	0	0	0	0
p-Dimethylaminobenzaldehyde	0	0	0	0	0	0	0	0	0	0
Ferric ferricyanide-Fe (III)	0	0	0	0	0	0	0	0	0	0
Thioglicolate-potassium ferricyanide-Fe (III)	0	0	0	0	0	0	0	0	0	0

0: negative; 1: weak; 2: moderate; 3: strong; C: control; 15, 10, 8.5 and 5: used concentration in mg/l; NT: not tested; Ort: ortochromasia; *: alternate regions of strong presence and absence.

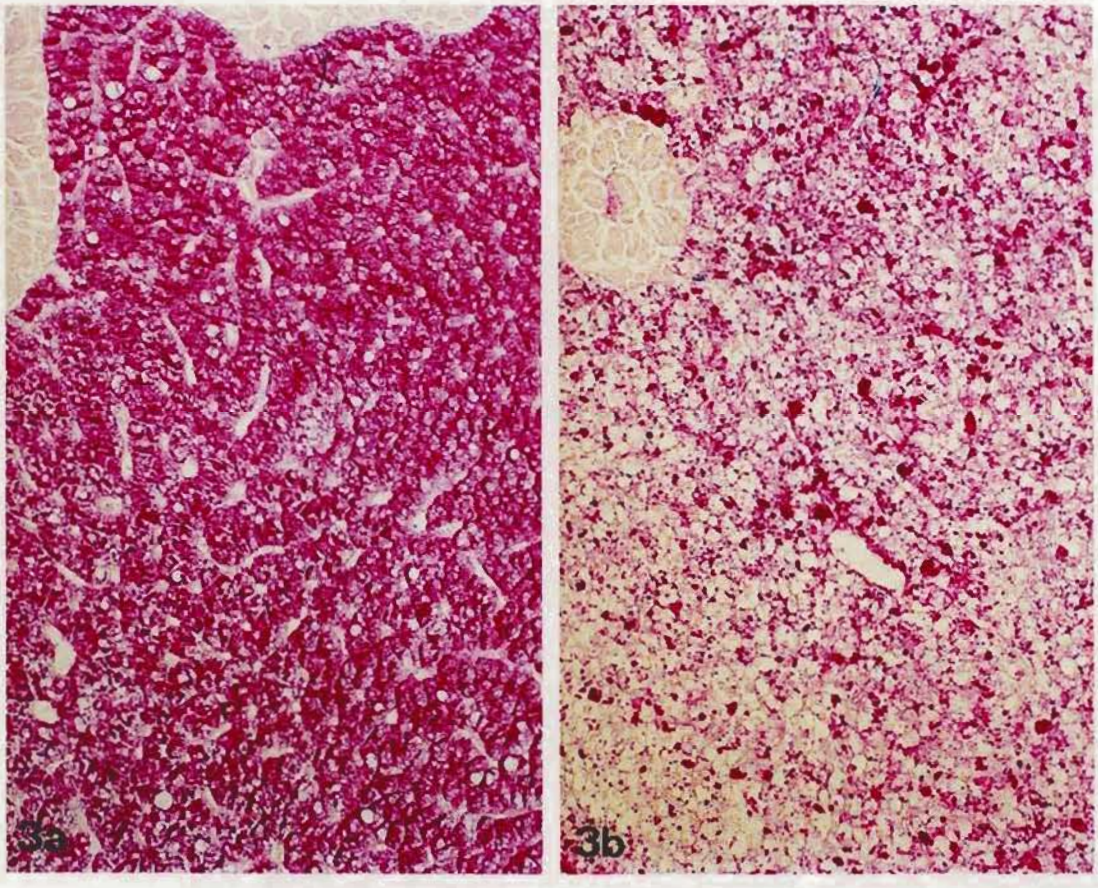

Fig. 3. Liver-pancreas of *sparus auratus*, L. stained with PAS. **a.** Control. **b.** Group B. x 250

Neutral mucopolysaccharides were shown only weakly in liver parenchyma. The histochemical results showed very low proteins in general and siderophile protein content both in hepatocytes and pancreatic cells.

Group B

At 10 mg/l there was a greater loss of the radial arrangement of hepatocytes. There was breakage of some pancreatic tubules with spillage of blood cells into the hepatic parenchyma. The majority of glands contained no zymogen and those that did had very little. In hepatic tissue, neutral mucopolysaccharides showed alternate regions of strong presence and absence (Fig. 3b). The behaviour of proteins was similar to that in specimens exposed to 15 mg/l.

Group C

At 8.5 mg/l, the radial arrangement of hepatocytes was completely lost and they were retracted. The zymogen content of pancreatic cells was less than in controls, but greater than in fish exposed to 10 mg/l (Fig. 1b). Neutral mucopolysaccharides were not observed, and the content of proteins in general and siderophile proteins (Fig. 5b) was less than in controls.

Group D

At 5 mg/l, hepatocytes were contracted which altered their radial arrangement (Fig. 2b). There were some glands containing differing quantities of zymogen and others that contained none. Neutral mucopolysaccharides were not observed. Proteins in general fall only slightly compared to controls (Fig. 4b). Siderophile proteins were less than in controls.

Discussion

The LC50 determined was higher than that cited by authors working with other fish (Marchetti, 1964; Laroche et al., 1972; Quevedo et al., 1984; Aguilera and Huq, 1982). The gilthead may therefore be considered to be relatively resistant to SDS, as suggested by Gomez et al. (1984), due to its pelagic nature, but perhaps also due to the idiosyncrasy of the species with respect to this agent. This is of great importance because fish that have been exposed to toxic concentrations of detergent are eaten.

Mann (1972) reached the conclusion that surface tension values of less than 40 mN/m are lethal for the

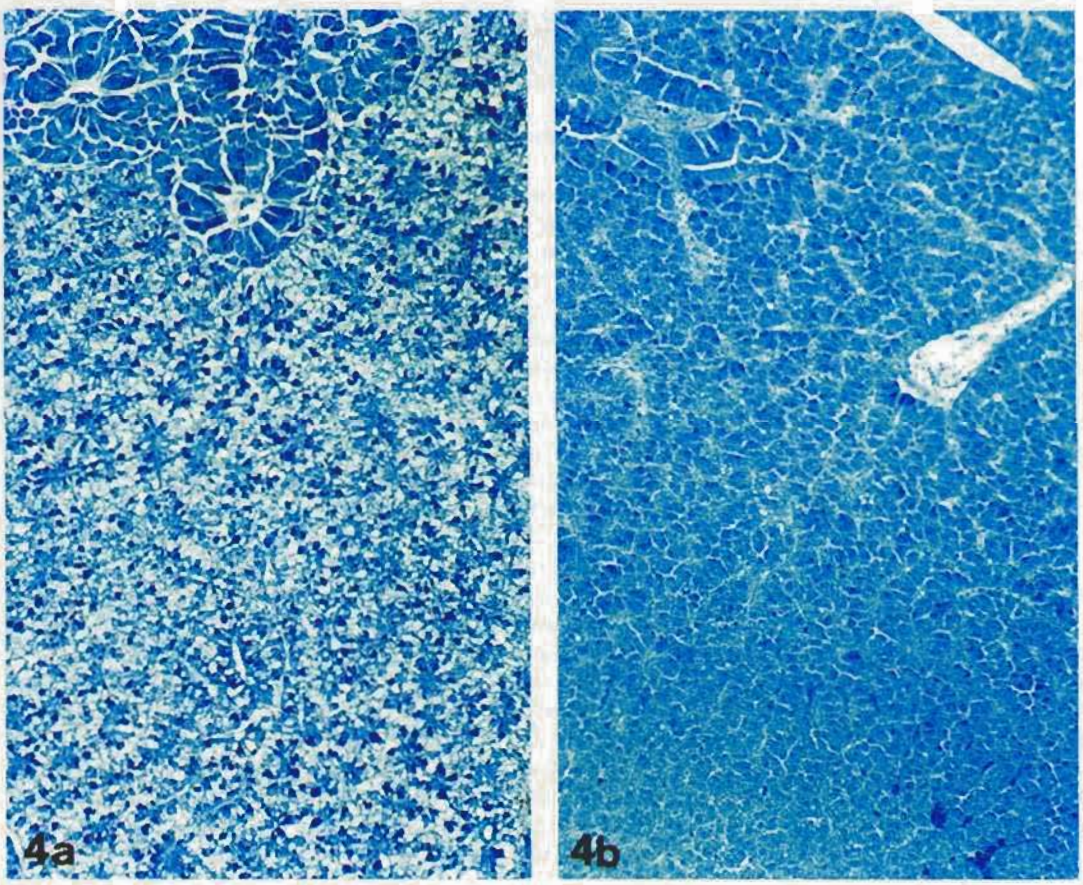

Fig. 4. Liver of *sparus auratus*, L. stained with Bromophenol blue. **a.** Control. **b.** Group D. x 250

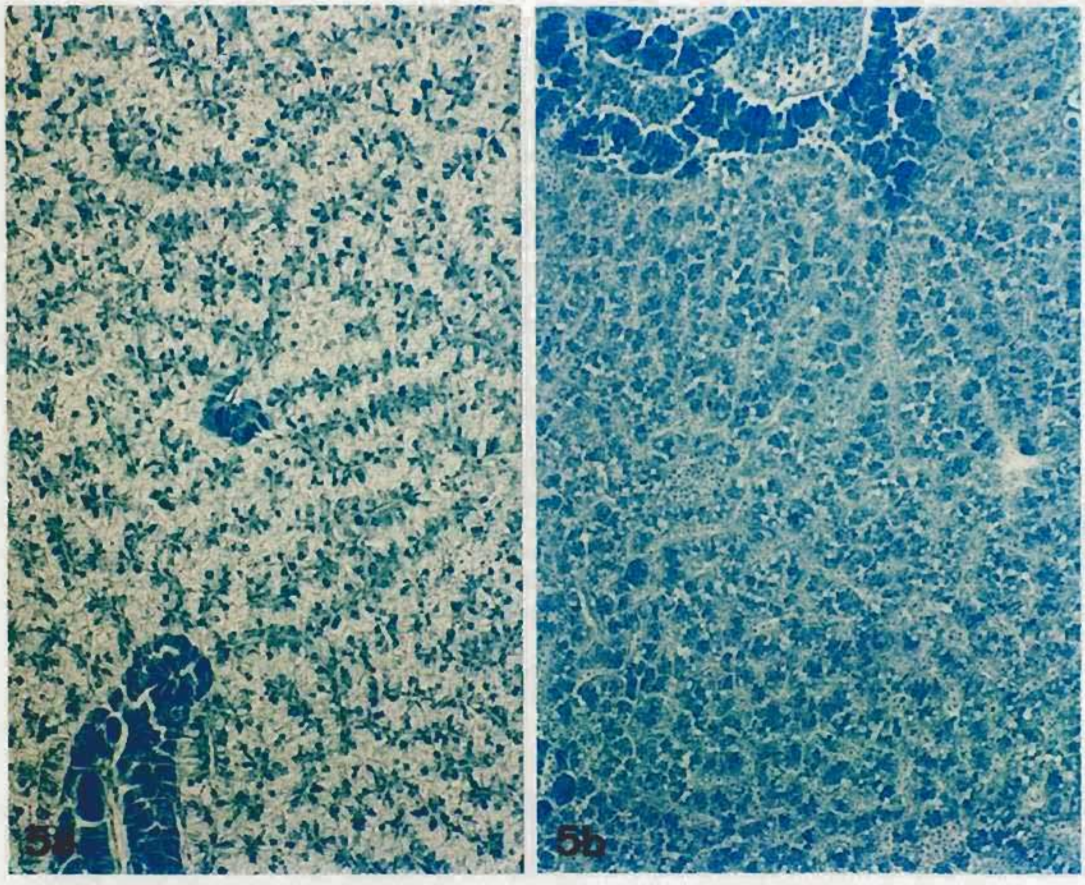

Fig. 5. Liver of *Sparus auratus*, L. stained with Hartig Zacharias method. **a.** Control. **b.** Group C. x 250

majority of species. Surface tension values of 50 mN/m are dangerous to the normal development of marine fauna (Bock, 1965). Marchetti (1964) however, suggests that there is not always a simple relationship between surface tension and toxicity.

In agreement with Sprague (1976) and Mallat (1985), this work does not confirm a single cause of death. Rather, the fall in surface tension, tissue destruction and effects at organ level are co-contributors to the death of giltheads exposed to SDS. Although fish internal organs have no initial direct contact with the detergent, it may be supposed that it eventually reaches them via the blood. We cannot confirm hepatic dysfunction as the cause of death, but the results do show serious hepatic damage and alterations in proteins and carbohydrates.

Our observations show that time of death and extent of lesions are related to the dose received and length of exposure, and that surface tension decreases with increasing concentrations of detergent. The effects of strong concentrations of detergent would be especially important in fishing areas.

It is presumed that the observed histochemical and morphological changes reveal disturbances that may have damaging effects upon determined biological functions.

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Accepted March 10, 1995