ORIGINAL PAPER

Eur. J. Histochem.
39, 141-148, 1995

Morphological and histochemical changes caused by sodium dodecyl sulphate in the gills of giltheads (*Sparus aurata, L.*)

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Accepted 15/03/1995

Key words: histochemistry

SUMMARY

An investigation was made into the biological effects of the anionic detergent sodium dodecyl sulphate (SDS), on the gills of giltheads (*Sparus aurata, L.*). The fish were exposed to concentrations of 5, 8.5, 10 and 15 mg/l SDS. The surface tension acquired at each concentration was determined and the LC50 calculated. Serious morphological damage to the gills and changes in protein and carbohydrate molecules (demonstrated by histochemical techniques) were observed. The degree of these alterations was dependent upon the SDS concentration and the length of time of exposure. It is suggested that changes in the gills lead to respiratory dysfunction and that this may be one of the major causes of death.

INTRODUCTION

Marine pollution is intimately related to socio-industrial development (Bogan, 1955; Prat and Giraud, 1964; Swedmark *et al.*, 1971; Flores *et al.*, 1979). However, the damaging effect of this pollution on marine fauna has not been well evaluated (Schmid and Mann, 1961; Lang, 1967; Granmo, 1971; Mahajan and Singh, 1972; Abel, 1974, 1976; Cotta and Rossaro, 1975; Brown *et al.*, 1978; Fukuda, 1982). In particular, little is known about the histochemical changes such pollution may induce. Marine pollution is caused in part by the release of detergents. Some 70% of industrial production of detergents corresponds to anionic detergents (García Dominguez, 1986), the family to which SDS belongs.

The gilthead (*Sparus aurata, L.*), a common species of the Mediterranean and Western Atlantic, was used to evaluate the effects of SDS. This species is pelagic, euryhaline and very sensitive to any fall in the concentration of dissolved oxygen (Arias, 1976, Arias *et al.*, 1984). This last characteristic makes it a useful tool in the study of pollutants that effect the oxygenation of water or that act on the respiratory system.

Fish gills are organs for respiratory gas exchange, osmoregulation, excretion of nitrogenous waste products and acid-base regulation. The structural complexity of the branchial epithelium reflects this multifunctional character (Franchini *et al.*, 1994). Many water pollutants are known to affect branchial structure, including detergents (Mallat, 1985). As primary site of respiration and osmoregulation, gills are the main target for the aquatic toxicant and is

the most seriously affected organ (Misra *et al.*, 1985). The probable causes of detergent toxicity include the absorption of the agent itself by marine organisms and, the induced reduction in the surface tension of their medium which limits their access to dissolved oxygen, and the osmoregulation process. In this investigation, changes in certain bio-macromolecules (as determined by histochemical methods) were correlated with the surface tensions acquired at each concentration of SDS.

MATERIALS AND METHODS

The fish used in this investigation were sixty five juvenile giltheads, *Sparus aurata, L.*, 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 lots, A, B, C, and D, which were exposed to concentrations of 5, 8.5, 10 and 15 mg/l of SDS Merck, Spain) respectively, until 50% of fish in each treatment lot died. Each lot was maintained in a PVC tank containing 200 litres of sea water, whose characteristics were: salinity 30%, pH 7.4, temperature 16-18^{0}C, dissolved oxygen 8-8.6 mg/l, hardness 100 mg $CaCO_3$/l, surface tension 72.7 mN/m, no contamination with heavy metals or microorganisms.

The LC50 causing death in a period of 96 hours was determined (Sprague, 1976; Loomis, 1982). To avoid variations in detergent concentrations, water and detergent 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 lots, lot C was further measured at 6h and lots A and B at 12h. After the animals died from exposure to the detergents, their gills 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 paraffin wax with a mean fusion point of 54-56^{0}C. Sections were cut at 5μm.

Harris's haematoxylin and acetic eosin, and Harris's haematoxylin-VOF (Gutierrez, 1961, 1967) were employed as general stains. The histochemical techniques used were: Schiff reagent (Schiff, 1865), periodic acid-Schiff [PAS] (McManus, 1948), diastase-PAS (Lillie and Greco, 1947), alpha amylase-PAS (Lillie and Greco, 1947), clorhydric hydrolysis-PAS (Martoja and Martoja-Pierson, 1970), alcian blue pH 2.5 (Lev and Spicer, 1964), methylation-alcian blue pH 2.5 (Lillie, 1958), methylation-saponification-alcian blue pH 2.5 (Lillie, 1958), hyaluronidase-alcian blue pH 2.5 (Quintarelli, 1963), alcian blue pH 1 (Lev and Spicer, 1964), alcian blue pH 0.4 (Lev and Spicer, 1964), hyaluronidase-alcian blue pH 0.4 (Pearse, 1960), toluidine blue (Martoja and Martoja-Pierson, 1970), bromophenol blue-Hg (Chapman, 1971), Hartig Zacharias method (Martoja and Martoja-Pierson, 1970), nihydrin-SCHIFF (Pearse, 1960), nitrosation-ninhydrin-SCHIFF (Pearse, 1960), 1-2 naphtoquinone-4-sulphonic acid sodium salt [NQS] (Lillie *et al.*, 1971), benzil-NQS (Lillie *et al.*, 1971), p-dimethylaminobenzaldehyde (Barka and Anderson, 1963), ferric ferricyanide-Fe [III] (Chevremont and Frederic, 1943), N-ethylmaleimide-ferric ferricyanide-Fe [III] (Chevremont and Frederic, 1943), thioglicolate-potasium ferricyanide-Fe [III] (Chevremont and Frederic, 1943).

RESULTS

The LC50 was 6.1 mg/l of SDS at 96 hours. The surface tension and time to death resulting at each concentration (Table I) is outlined below: Lot A: 56.0mN/m, 58.2mN/m, 250h; Lot B: 54.3mN/m, 56.1mN/m, 12h; Lot C: 53.3mN/m, 53.9mN/m, 6h; Lot D: 49.9mN/m, 30min.

Our observations of gill filaments showed (Figs. 1.a, 2.a, 2.c) an organization similar to that described for other teleostean gills (Laurent and Dunel, 1980; Hughes, 1984; Laurent, 1984; Franchini *et al.*, 1994). Gill filaments or primary lamellaes gave rise to rows of secondary or respiratory lamellae including the interlamellar region. The primary and secondary lamellae were covered by the primary and secondary epithelia, respectively, which was formed by different cell types: pavement, chloride and pillar cells; but not mucous cells, which are not observed in *Sparus aurata, L.*

Observed morphological changes included:

Lot A: (Fig. 1.b) at 5mg/l there is shortening of lamellae (both longitudinal and lateral) with a ten-

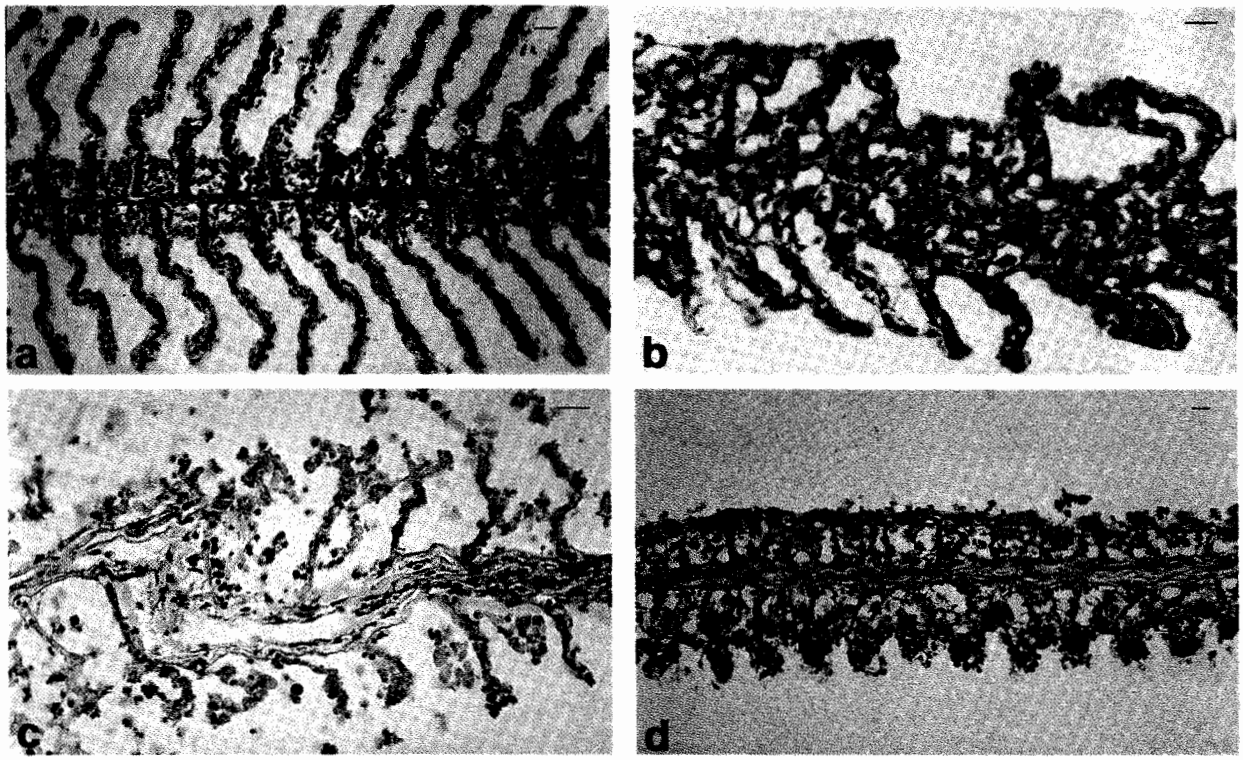

Fig. 1 - a) Control gill filaments of *Sparus aurata, L.* Harris's haematoxylin-VOF. Bar, 10μm; **b)** Gill filaments of *Sparus aurata, L.* treated with dose of 5 mg/l of SDS, tendency of the lamellae to fuse. Harris's haematoxylin-VOF. Bar, 10μm; **c)** Gill filaments of *Sparus aurata, L.* treated with dose of 8.5 mg/l of SDS, shortering of the gill filaments and loss of epithelial cells, Harris's haematoxylin-VOF. Bar, 10μm; **d)** Gill filaments of *Sparus aurata, L.* treated with dose of 15 mg/l of SDS; fusion of the lamellae and loss of filaments epithelium. Harris's haematoxylin-VOF. Bar, 10μm.

Table I
Time to death and the surface tension resulting at each concentration

SDS CONCENTRATION	TIME TO DEATH	SURFACE TENSION		
		Initial	6 hours	12 hours
5 mg/l	250 hours	56.0 mN/m		58.2 mN/m
8.5 mg/l	12 hours	54.3 mN/m		56.1 mN/m
10 mg/l	6 hours	53.3 mN/m	53.9 mN/m	
15 mg/l	30 min	49.9 mN/m		

dency of the lamellae to fuse. Although blood vessels appear normal there is hemorrhage of lamellar capillaries.

Lot B: (Fig. 1.c) at 8.5mg/l there is longitudinal contraction (shortening) of the gill filaments and almost total loss of filament epithelium. The loss of lamellar and filament cells is very great with only the filamental cartilage remaining.

Lot C: at 10mg/l lamellar fusion and epithelial destruction is so extensive that only the branchial fila-

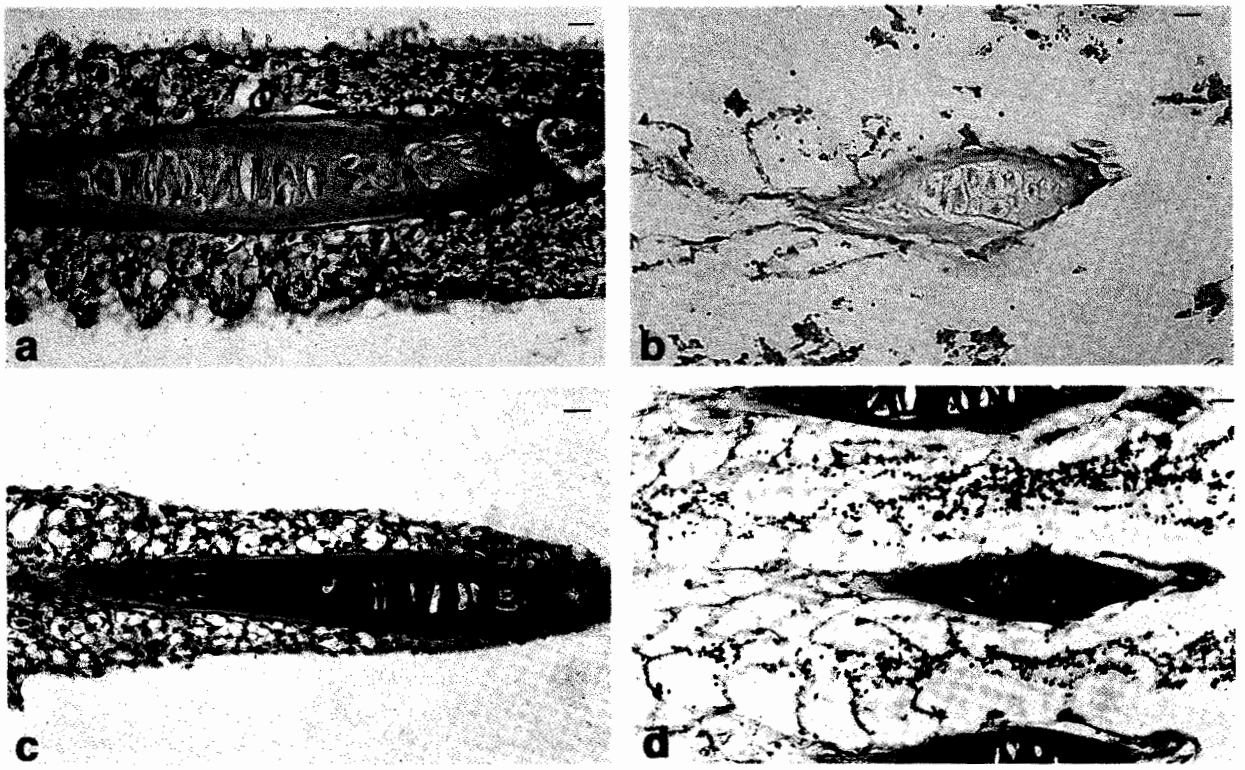

Fig. 2 - a) Control gill filaments of *Sparus aurata, L.* Bromophenol blue. Bar, 10μm; **b)** Gill filaments of *Sparus aurata, L.* treated with dose of 10 mg/l of SDS, showing decreased Bromophenol blue positivity, Bromophenol blue. Bar, 10μm; **c)** Control gill filaments of *Sparus aurata, L.* Toluidine blue. Bar, 10μm; **d)** Gill filaments of Sparus aurata, *L.* treated with dose of 8.5 mg/l of SDS; metachromasia in mature cartilage is not altered to exposure to SDS. Toluidine blue. Bar, 10μm

ments remains; there is a great loss of filament epithelium.

Lot D: (Fig. 1.d) at 15mg/l fusion of the lamellae is seen with destruction of the gill filaments, which results in their shortening. There is retraction of the gill filaments and loss of filament epithelium. Vascular dilation is observed and there are blood cells between the gill filaments.

The results of histochemical analysis are shown in Tables II and III. A noticeable feature is the general decrease in acidic mucopolysaccharides and proteins (Figs. 2.a, 2.b). Toluidine blue reveals metacromasia in mature cartilage which is not altered by exposure to SDS (Figs. 2.c, 2.d). A further point of interest is the change of staining of epithelial cells after exposure to SDS. After staining with haematoxylin and eosin, only haematoxylin is seen in specimens subjected to 8.5, 10 and 15 mg/l. Staining with haematoxylin-VOF gives brown colouration at 5mg/l (there are practically no epithelial cells), green at 10mg/l and brown at 15mg/l (Figs. 1.a, 1.b, 1.c, 1.d).

DISCUSSION

SDS causes serious morphological damage to the gills of giltheads which undoubtedly will hamper respiratory and osmoregulatory function of the gills. The changes observed in this investigation agree with those described in *Rita rita* when exposed to DSBB (Roy, 1988a). Similarly, Roy (1988b, 1989) observed changes in certain proteins and carbohydrates. Moreover, a fall in the activity of respiratory enzymes was reported in *Heteropneustes fossilis* when exposed to Na-alkyl-benzenesulphonate (Zaccone, 1985), demonstrating that agent's ability to interfere with respiratory mechanisms.

We did not observe epithelial cells lift, hypertrophy epitelia, hyperplasia epitelial, clavate-globate lamellae, nor congested blood cells in lamellae (Mallat, 1985).

The progressive loss of colour over the range of gill epithelial cells stained with haematoxylin and eosin -specially in the case of eosin- could be due

Table II
Histochemical reactions of carbohydrates in the gill of *Sparus aurata, L.* exposed to different concentrations of S.D.S.

	Lamellar epithelium					Filament cartilage				
Reaction	Ctrl	15	10	8.5	5	Ctrl	15	10	8.5	5
SCHIFF	0	0	0	0	0	0	0	0	0	0
PAS	0	0	0	0	0	1	1	1	1	1
Diastase-PAS	NP	NP	NP	NP	NP	1	1	1	1	1
Alpha amylase-PAS	NP	NP	NP	NP	NP	1	1	1	1	1
Clorhydric hydrolysis-PAS	0	0	0	0	0	1_b	1_b	1_b	1_b	1_b
Alcian blue pH 2.5	0	0	0	0	0	2_b	1_b	1_b	1_b	1_b
Methylation-alcian blue pH 2.5	NP	NP	NP	NP	NP	0	0	0	0	0
Methylation-saponification-alcian blue pH 2.5	NP	NP	NP	NP	NP	1_b	1_b	1_b	1_b	1_b
Hyaluronidase-alcian blue pH 2.5	NP	NP	NP	NP	NP	1_b	1_b	1_b	1_b	1_b
Alcian blue pH 1	0	0	0	0	0	2_b	1_b	1_b	1_b	1_b
Alcian blue pH 0.4	0	0	0	0	0	2_b	1_b	1_b	1_b	1_b
Hyaluronidase-alcian blue pH 0.4	NP	NP	NP	NP	NP	2_b	1_b	1_b	1_b	1_b
Toluidine blue (metachromasia)	NO	NO	NO	NO	NO	Yes	Yes	Yes	Yes	Yes

Note: $_b$: chondrocyte border; 0, negative; 1, weak; 2, moderate; 3, strong. Crtl: control; 15, 10, 8.5, 5= concentration used in mg/l. NP: has not been performed.

to competition between stain components and amphiphilic molecules of the detergent for fixation sites. The change of hue towards brown (a mixture of acid fuchsin and light green) when using Harris' haematoxylin VOF could be due to a change in tissue integrity which prevents the retention of molecules of orange G, the smallest component of the stain.

The mechanisms by which detergents exert their effects are not yet understood (Helenius and Simmon, 1975). However, our results confirm the following results from the literature:

i. the fall in surface tension limits the access of the gill to dissolved oxygen. It is thought that the fall in surface tension induces the main cause of death (Prat and Giraud, 1964; Bock, 1965; Mann, 1972). Studies made by Mann (1972) conclude that surface tension values of less than 40mN/m are lethal for the majority of species. However, it has also been claimed that surface tension has little to do with the toxic effects of detergents on fish (Marchetti, 1964, 1965; Müller, 1980). The results of this work have shown a close relationship between detergent concentration, fall in surface tension and time to death.
ii. the loss of gill integrity is so serious that the fish may be expected to have died from asphyxia and the loss of osmotic and ionic regulation provoked by the destruction of the branchial epithelia, including the chloride cells (Lock and van Overbeeke, 1981; Wendelaar Bonga and van der Meij, 1989; Zaccone *et al.*, 1994; Franchini *et al.*, 1994).

Table III
Histochemical reactions on proteins in the gills of *Sparus aurata. L.* exposed to different concentrations of S.D.S.

	Lamellar epithelium					Filament cartilage				
Reaction	Ctrl	15	10	8.5	5	Ctrl	15	10	8.5	5
Bromophenol blue-Hg	3	2	1	1	1	1	1	1	1	1
Hartig Zacharias method	1	1	1	1	1	1	1	1	1	1
Ninhydrin-SCHIFF	0	0	0	0	0	0	0	0	0	0
Nitrosation-ninhydrin-SCHIFF	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
NQS	0	0	0	0	0	0	0	0	0	0
Benzil- NQS	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
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
N-ethylmaleimide-ferric ferricyanide-Fe (III)	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Thioglicolate-potasium ferric-yanide-Fe (III)	0	0	0	0	1	1	1	1	1	1

Note: 0, negative; 1, low; 2, moderate; 3, high. C: control; 15, 10, 8.5, 5= concentration used in mg/l. NP: has not been performed.

Finally, the LC50 at 96h. of juvenile giltheads was found to be 6.1mg/l SDS. This dose is noticeably higher that that found for other species of fish by other authors (Gomez *et al.*, 1984). This result suggests that the gilthead is relatively resistant to this detergent.

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