Acute toxicity of anionic surfactants sodium dodecyl sulphate (SDS) and linear alkylbenzene sulphonate (LAS) on the fertilizing capability of gilthead (*Sparus aurata* L.) sperm

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Summary. In the present work we have evaluated and compared the acute toxicity of two anionic surfactants, Sodium Dodecyl Sulphate (SDS) and Linear Alkylbenzene Sulphonate (LAS) on the fertilizing capability of gilthead *Sparus aurata* L. sperm. The criterion used to judge exposure effectiveness was fertilization success.

Spawned eggs and sperms were collected from adult giltheads. Sperms were dosed separately with different concentrations of SDS and LAS for 60 minutes. After this period, sperms and eggs were combined for 20 minutes during which fertilization took place. Finally, the number of fertilized eggs were counted and recorded to estimate the percentage of fertilization.

Exposure to SDS and LAS concentrations of 0.3, 0.6, 1.5, 3 and 6 mg/L for 60 minutes caused a significant inhibitory effect on fertilization success in gilthead *Sparus aurata* L.. In addition, the EC50 value for gilthead fertilization after sperm exposure was found to be 2.8 mg/L and in the case of LAS it was of 2.6 mg/L.

The comparison of the results from SDS and LAS shows that the latter has a stronger negative effect on sperm viability than SDS.

Key words: Pollution, Surfactant, Sperm, Fertilization, *Sparus aurata*

Introduction

The wide variety of processes in which surfactants are incorporated has resulted in a spectacular increase in their consumption, which has grown from about 13×10^6 tons in 1977 to 18×10^6 tons in 1995 (Granados, 1996). Of this total, about 1.5 million tons correspond to anionic surfactant linear alkylbenzene sulphonate (LAS), which means that this is the world's leading type of surfactant in terms of consumption volume (Perales et al., 1999).

Sodium dodecyl sulphate (SDS) is also a widely commercialized anionic surfactant, whose surface active properties make it important in hundreds of household and industrial cleaners, personal care products and cosmetics. It is also used in several types of industrial manufacturing processes as a delivery aid in pharmaceuticals and in biochemical research involving electrophoresis (Singer and Tjeerdema, 1993). In addition, anionic surfactants are important components of dispersants, whose use in oil-spill clean up programs has been contentious for many years (Hatcher and Larkum, 1982).

Due to their widespread use, there has been an increasing concern about the environmental behavior of these compounds. Domestic and industrial effluents containing nonbiodegradable surfactants still flow into natural waters, causing profound effects on the aquatic environment (Muramoto et al., 1996).

Faunal and chemical monitoring has frequently been used to assess environmental quality (Martin and Richardson, 1995). The use of biota as an indicator of pollution is advantageous over chemical analysis as they are ecologically realistic (Pocklington and Wells, 1992). In this connection, these days it is of great importance to evaluate the effects of pollution on fish both for environmental protection and for socio-economic reasons (Lin and Hwang, 1998).

Gilthead *Sparus aurata* L. was used as a test species because it is a widespread fish in the SouthAtlantic coast of Spain with a great economic-commercial interest in the fishing industry. Besides, it is commonly exploited in extensive aquaculture production of some southern European countries such as Spain (Arias et al., 1976).

It should also be mentioned that aquatic organisms such as gilthead are external fertilizers which shed their gametes into waters that may contain putative environmental contaminants. Furthermore, in fish,

opposite to mammals, large volumes of sperm and thousands of oocytes can be easily obtained for in vitro fertilization studies. For the reasons already mentioned, fish species such as gilthead *Sparus aurata* L. can also be a useful lower vertebrate model in studies of the effects of xenobiotics on male fertility (Ciereszko and Dabrowsky, 2000).

Acute toxic effects of anionic surfactants on aquatic species have been studied mainly in juvenile and adult life stages (Okuwosa and Omoregie, 1995; Ribelles et al., 1995a-c; Rosety et al., 1997, 2000). The effects of this phenomenon on the early developmental stages have received little attention (Ankley and Burkhard, 1991; Kusk and Petersen, 1997). It is generally accepted that earlier life stages of marine organisms are found to be more sensitive to pollutant stress than adults (Mohan et al., 1983; Kwain and Rose, 1985; Dave and Xiu, 1991).

Accordingly, the election of sperm as test organism was based on the evidence that it is among the most sensitive life stages in fish. In addition, it may be considered as a sensitive and readily available species-specific bioindicator of pollution (Kime, 1995). Further, the sperm cell toxicity test requires only 60 minutes of toxicant exposure whereas other marine embryo and larval development tests require 2 to 4 days of exposure, both having similar sensitivities (Dinnel, 1995).

For the reasons already mentioned, the present investigation was carried out to evaluate and compare the acute toxicity of several concentrations (0.3, 0.6, 1.5, 3 and 6 mg/L) of anionic surfactants SDS and LAS on the fertilizing capability of gilthead sperm.

Materials and methods

Adult giltheads *Sparus aurata* L. were collected from a culture unit at San Fernando (Cadiz, Spain). We selected three reproductive females, varying in weight between 4 and 4.5 kg whose eggs had shown previously a 90% fertilization success or more, to contribute to the pool of eggs for each bioassay. According to Kadmon et al. (1985) spawning was artificially induced by a photoperiod regime (8 hr light and 16 hr dark photoperiod). Breeders were maintained at a constant temperature of 20±1 °C and at 33-35 ‰ salinity.

Released eggs were filtered through a relatively coarse screen to remove debris and washed 3 times in 300 mL of seawater before being used in the bioassay. The egg stock was diluted to 2000 eggs/mL using a Sedgwick-Rafter chamber to count them.

Three males were chosen to collect the sperm stock for our bioassay. It was kept at 4 °C in a dry undiluted state until the beginning of the experiments. Samples contaminated with urine or blood were discarded. Sperm viability was assessed by observing under a microscope their mobility in seawater. Sperm stock was diluted to 7×10^7 sperms/mL using a Neubauer chamber.

To prevent the adhesion of sperms, the tubes were precoated by dipping in 1% polyvinyl alcohol (average molecular weight 30000-70000; Sigma) solution and dried at 60 °C (Perchec et al., 1995).

In the experiments on the determination of surfactant toxicity for sperms, 0.1 mL of sperm suspensions were exposed for 1 hour in individual test tubes containing either SDS or LAS in concentrations of 0 (controls), 0.3, 0.6, 1.5, 3 and 6 mg/L.

After this period, 1 mL of the unexposed egg suspension was added to each test tube. After 20 minutes exposure during which fertilization took place, the tests were stopped by adding 2 mL of a 10% solution of formaldehyde in seawater.

Finally, fertilization rate in a 100-egg subsample per replicate was analyzed under a microscope. It may be noted that for each compound, the series of concentrations were tested in triplicate.

The criterion used to judge exposure effectiveness was fertilization success after being combined with unexposed gilthead eggs. Fertilization, defined as the presence of a fertilization envelope, was assessed by microscopic observation. While Dinnel et al. (1982) considered unfertilized eggs if they had partially formed membranes, we considered them fertilized since the test endpoint is the evaluation of the fertilizing capability of the sperm. Partial membranes indicate the existence of enough viable sperm to fertilize the eggs, in spite of the egg's inability to raise the whole fertilization membrane (Nipper et al., 1993).

Stock solutions of SDS and LAS were diluted with sperm-free seawater in order to obtain the desired concentrations. The seawater used for dilution and control tubes was filtered through a 0.5 μm cellulose filter prior to being used. Its physiological characteristics were as follows: salinity 33-35‰, pH 7.4, temperature 20 ±1 ºC, dissolved oxygen 8-8.6 mg/L, total hardness 100 mg CO_3 Ca/L, surface tension 72.7 mN/m and absence of heavy metals.

The test results were expressed as percentage fertilization (means ± SD). The "trimmed Spearman-Karber" method was performed in this toxicity test to estimate the median lethal concentrations (EC50) for SDS and LAS (Hamilton et al., 1977).

Results

Sperm/egg ratios of 3500 were selected for subsequent experiments to evaluate the effects of both surfactants on the sperm fertilizing capability. Using this ratio, fertilization rate in control was 90%. In addition, the potential problem of high sperm concentrations masking the toxicity of both surfactants was avoided.

All SDS and LAS tested concentrations caused a pronounced reduction in the fertilizing capability of sperms. The individual results of the several tests prepared with both toxicants are presented in Figure 1.

Percentage of fertilized eggs following 60 minutes exposure of *Sparus aurata* sperms to different concentrations (0.3, 0.6, 1.5, 3 and 6 mg/L) of SDS were 78, 72, 60, 43 and 7% respectively. Besides, the EC50 value for fertilization of giltheads after sperm exposure

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to SDS was found to be 2.8 mg/L.

Gilthead sperms exposed to LAS concentrations of 0.3, 0.6, 1.5, 3 and 6 mg/L for 60 minutes exhibited fertilization rates of 76, 68, 54, 37 and 3 respectively. The EC50 value with LAS was of 2.6 mg/L.

Discussion

The toxicity of surface active agents to marine organisms is fairly well documented, as a result of its wide presence in industrial and urban discharges. The vast literature regarding the acute toxic effects of anionic surfactants on aquatic species have been focused mainly on juvenile and adult life stages (Mishra et al., 1985; Ribelles et al., 1995a-c; Rosety et al., 1997, 2000).

On the other hand, the effects of this phenomenon on free gametes have received little attention (Nipper et al., 1993; Weber et al., 1988).

In agreement with Harrison and Wallace (1990), fertilization success was used as the criterion of this investigation because of its potential vulnerability to external influences and for the importance of repeated successful spawning years for the long term maintenance of these populations in nature.

In the present study, an inhibitory effect of both anionic surfactants on fertilization success in *Sparus aurata* L., was evident. In this respect, gilthead sperms exposed to a SDS concentration of 6 mg/L for 60 minutes exhibited a fertilization rate of 7%. SDS concentration as low as 0.3 mg/L enormously depressed fertilizing capability of the sperm in gilthead. In the case

of LAS, fertilization success was significantly reduced to 76% at 0.3 mg/L and 3% fertilization occurred at 6 mg/L of LAS. High fertilization rates of 90% were recorded in controls.

Clear dose-response relationship for the reduction on percentage fertilization of *Sparus aurata* was observed for both toxicants. This relationship was also found by Vaschenko et al. (1999) on sperm of sea urchin *Anthocidaris crassispina* (Agassiz) exposed to cadmium. Descriptively our results indicated that both surfactants presented different toxicity, fertilization success being more depressed after exposure to LAS than in the case of SDS. This fact was specially evident at high concentrations. In this respect, at 6 mg/L, percentage fertilization for SDS was of 7% and for LAS was of 3%. At this concentration, LAS seemed to be twice as toxic than SDS (percentage fertilization is compared).

In a previous study, Weber et al. (1988) reported the toxicity of anionic surfactant SDS on sea urchin *Arbacia punctulata* sperm. The EC50 in this experience was found to be 3 mg/L of SDS. Similarly, the EC50 presented by Nipper et al. (1993) for echinoderm *Lytechinus variegatus* sperm exposed to SDS was of 2.9 mg/L. Although differences in experimental procedures and conditions between the studies urge to proceed with caution, these data suggest that the sensitivity of gilthead sperm to SDS was in the same range of that of both echinoderm species.

It has also been well documented that the early life stages of aquatic organisms were much more sensitive to pollution pressures than juveniles and adults (Mance,
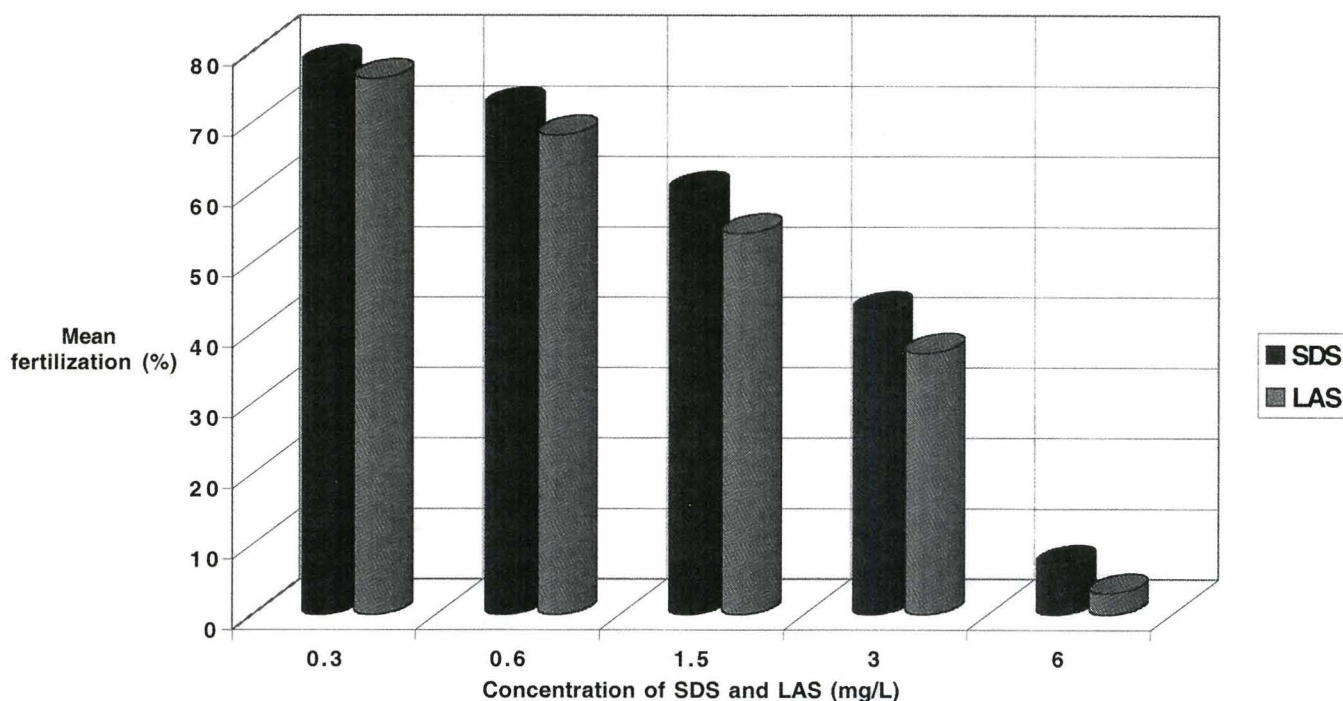

Fig. 1. Effects of SDS and LAS on fertilization success in *Sparus aurata* at differetn concentrations. Each value is an average of three experiments.

1987). In this connection, Ribelles et al. (1995 a-c) reported that the LC50 at 96 h for juvenile giltheads with SDS was found to be 6.1 mg/L. In our experience, at 0.6 mg/L of SDS for 60 minutes, percentage fertilization was reduced to 7% and even concentrations as low as 0.3 mg/L of SDS reduced enormously fertilization rate. It serves to illustrate that sperms are also more sensitive to surfactants than older life stages.

The question arises how surface active agents exert their toxic action on gilthead sperms. It is thought that the fall in the surface tension induced by surfactants may be the main cause of death (Prat and Giraud, 1964). However, it has also been claimed that surface tension has little to do with the toxic effects of surfactants on fishes (Muller, 1980).

In a previous study, Thorhaug (1992) reported that surfactants may disrupt biological membranes and subcellular organelles as well as disturbing the function of some enzymes.

While the mechanisms of surfactant toxicity on gilthead sperm remain to be ascertained, potential targets of its toxicity may very well include decrease in surface tension, destruction of biological membranes and subcellular organelles and the alteration of the function of some enzymes, etc.

Although extrapolation from the laboratory to the field requires caution, the results of this work suggest that relatively low concentrations of SDS and LAS critically disrupt reproductive processes of this species. This fact may lead to a long-term decline or eventual extinction of gilthead populations in nature when they are located close to effluents that either are untreated or receive inadequate secondary treatment. It is also quite important because gilthead *Sparus aurata*, L. constitutes an important link in the food chain and its death via exposure to surfactants may imbalance the litoral ecosystem.

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