Cryptocaryon irritans and Enteromyxum leei, two threats for the culture of Diplodus puntazzo in the Mediterranean

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Abstract
Cryptocaryon irritans and Enteromyxum leei are pathogenic parasites which are known to affect many different fish species. The severe effects of these pathogens on the first tentative cultures of the sharp snout seabream in the Spanish Mediterranean have compromised the development of the local production. The records of these pathological episodes are used to illustrate the apparent recent expansion of these parasites in the Mediterranean.

Introduction
The sharp snout seabream (Diplodus puntazzo) is a sparid fish recently introduced in the Mediterranean aquaculture (Italy, Greece and Turkey). This species is valued in the markets of the Mediterranean countries showing very good culture prospect in experimental conditions (Abellan & García-Alcázar, 1995; Caggiano et al., 1993; Favaloro et al., 2002). Despite the brief history of its culture, several potentially severe pathogens have already been reported, namely Fungi (Candida spp., Ichthyophoniasp.), Protista (Trichodinasp., Amyloodiniumsp.), Myxozoa (Enteromyxum leei) and Monogenea (Gyrodactylus sp., Atrispinum salpae, A. seminalis and Sparicotyle chrysophrii) (Athanassopoulou et al., 1999; Di Cave et al., 1998; Madlineo, 2003; Rigos et al., 1998; Tampieri et al., 1999; Zaccone & La Ferla, 1988-1989). The culture of the gilthead seabream (Sparus aurata) represents the opposite situation, as it constitutes the majority of the production in Mediterranean aquaculture, with a long farming tradition. The settling of the culture of S. aurata has involved both a precise technical experience and a wide knowledge on diverse biological aspects of this fish species together with its pathologies and treatments. Many of the diseases of S. aurata are shared with other sparid species like the sharp snout seabream, probably due to the close proximity of the cultures and because the easy transmission between close phylogenetically related hosts (Golomazou et al., 2006; López-Román & De Armas-Hernández, 1973; López-Román & Guevara Pozo, 1979; Di Cave et al., 1998).

One of the interests in farming new fish species like D. puntazzo results from the
diversification policies of governments and institutions in order to increase the consumer offer and to avoid the “monocultures”, as in the case of S. aurata or also of Dicentrarchus labrax in the Mediterranean (CIHEAM, 1995; ICES, 2003; JACUMAR, 2004). The development of these alternative cultures requires the knowledge of potential diseases, especially when already established cultures of other fish species are susceptible to the same pathogens.

The present paper reports recent severe outbreaks of 2 parasites, Cryptocaryon irritans (Ciliophora) and the myxozoan Enteromyxum leei (Myxozoa), in the first culture experiences of D. puntazzo developed in the Spanish Mediterranean. These and other episodes previously reported appear to show an apparent expansion of these parasitoses both in wild and cultured Mediterranean fish species.

Material and methods
The sharpsnout seabream in this study were obtained from 3 different facilities along the Spanish Mediterranean coast; inshore concrete tanks of the Spanish Institute of Oceanography (SOI) in Mazarrón (37°31’N-37°34’N, 1°13’W-0°18’W), sea-cages and tanks in San Pedro del Pinatar (37°48’N-37°51’N, 0°44’W-0°45’W) and sea-cages in Gandia (38°53’N-39°05’N, 3°15’W-0°01’E) (Figure 1).
Fish from Mazarrón were analysed in 2000 and the stock had been imported from a parasite-free Italian hatchery as fingerlings (1-5 g) and reared in inland facilities. The samples from San Pedro del Pinatar and Gandía were analysed in 2005 and derived from the same transport tank from another parasite-free Italian hatchery, in March 2004.

In these facilities the abnormal behaviour and aspect of the animals were routinely observed and mortalities were recorded. Well preserved, freshly deceased animals or moribund fish, over-anesthetised by Eugenol, were collected for their analysis. Fish were examined for macroscopical changes by eye and light microscopically at x10-x80. Smears of skin, fins and gills were examined at x100-x1000 magnification. Subsequently, fish were dissected in order to perform a parasitological analysis of the organs. Fresh smears of the rectum were taken and observed under light microscope, using bright field and differential interference contrast techniques. Moreover, scrapings of the posterior intestinal mucosa were stained using Diff-Quik (Medion Diagnostics AG). Samples of the digestive tract (stomach, intestine and rectum), liver, gallbladder, haematopoietic organs and gills were fixed and processed for routine histology. Individuals of other fish species from the same infected installations were also analysed. Substrate samples from the tanks were also collected with a siphon and analysed.

Results
In September 2000, 59 mortalities were reported from 1+ sharpnout seabreams (324-410g) reared in the inshore facilities of the SOI in Mazarrón. 100% of the fishes in a single affected tank died within 15 days after the first signs of disease were reported. All sharpsnout seabream were heavily infected by \textit{C. irritans}. It is important to notice that other fish species in the same facilities, e.g. the greater amberjack \textit{Seriola dumerili}, were also seriously affected. In fact, 93% of 100 greater amberjacks of 1+ to 3+ ages died after 30 days from the beginning of the first symptoms. External signs of cryptocarionosis were not evident in the sharpsnout seabream held in the tanks. However, the behaviour of the affected fish was altered, showing loss of appetite and frequent scratching with the tank walls. No white spots in the skin could be observed by naked eye. Skin and fin petechiae and ulcerations were abundant in the fish (Figure 2a). Ulcerations associated to the frictions with the concrete tanks, were especially abundant in some severe cases. Gills were pale, with increased amounts of mucus. Both in the mucus and in the branchial epithelium white nodules of about 0.2 mm were distinguished by microscope. Numerous live trophonts and tomonts of \textit{C. irritans} were identified in the fresh smears of skin and gills (Figure 2b), both from ulcerations and from apparently healthy areas. \textit{C. irritans} tomonts were also detected in the substrate of the tanks. Tentative treatments with copper sulphate-citrate baths (1-2 ppm) were apparently not effective in any of the infected fish species. The disease only retreated when, in October, water temperatures decreased below 19°C.

Thereafter, pathologies were not reported in the cultures of \textit{D. puntazzo} in the Spanish Mediterranean until October 2005 when
several losses of sharpsnout seabream were recorded in 1+ fishes reared in sea-cages in San Pedro del Pinatar. At this facility a heavy infection with the myxozoan *E. leei* was diagnosed in all the fish stock. However, other fish imported at the same time from the same Italian supplier but maintained in other different installation (tanks of San Pedro del Pinatar and in sea-cages in Gandia) did not show so severe external damages. Fish in Gandia only showed some emaciated condition (n=20, mean weight 28.1 g; mean length 10.1 cm) when compared with those in the tanks in San Pedro del Pinatar (n=20, mean weight 76.7 g; mean length 13.4 cm). No evidence of infection of *E. leei* was observed during the analysis of the individuals reared in the tanks in San Pedro del Pinatar. In contrast, the 75% of the sharpsnout seabreams in sea-cages in Gandia were infected by the

Figure 2. Cryptocarion irritans and Enteromyxum leei in Diplodus puntazzo. A, Ulceration in the skin of *D. puntazzo* infected by *C. irritans*; B, *C. irritans* nodules in gills; C, detail of tomonts of *C. irritans* in a gill lamella (bar 300 µm); D, fresh preparation of sporogonic stage of *E. leei* showing two mature spores (bar 5 µm); E, mature spores of *E. leei* from a posterior intestinal scraping (bar 20 µm); F, transversal section of the posterior intestine of an infected *D. puntazzo*. Hematoxilin-eosin. (Bar 20 µm).
myxozoan. Examination of fresh smears of the rectal mucosa of 20 individuals from this location revealed the presence of semilunar spores typical for *E. leei* (Figure 2d). When hematoxilin-eosin stained sections were observed under light microscope the presence of presporogonic and sporogonic stages within the intestinal mucosa was confirmed (Figures 2e & 2f). No stages of the parasite were detected in any other tissues examined. Other fish species, which had accidentally entered in the infected cages in Gandia (2 *Liza aurata* and 4 *Trachurus mediterraneus*) were also found to have abundant *E. leei* spores within their rectal mucosa. Sharpsnout seabream from the sea-cages in both localities were stamped out to avoid a widespread to other cultured or wild fishes.

**Discussion**

Previous reports on sharpsnout seabream cultures have shown that this fish is highly susceptible to *C. irritans* and *E. leei* (Athanassopoulou et al., 1999; Caffara et al., 1999; Padrós et al., 2001; Montero et al., 2004; Sitjà-Bobadilla, 2004). Both parasites are harmful emergent pathogens in many species of the Mediterranean aquaculture as they are extremely virulent, with low host specificity and direct fish-to-fish transmission. In the cases here reported the parasites spread rapidly and fish died within a short time period. These properties make the eradication of these parasites very difficult and facilitate their contagion between to other cultures and animals in the wild. In fact, the parasites reported in this study apparently came from fish from wild populations. All sharpsnout seabream in the present cases came from parasite-free broodstock and apparently got infected when cohabitated with local fish species: infected fish in tanks of the same circulation system in the case of *C. irritans* in Mazarrón (possibly greater amberjacks), or infected wild fish associated with the sharpsnout seabream reared in sea-cages (as could be *L. aurata* and *T. mediterraneus* in the Gandía facilities) in the case of *E. leei*.

Historically, first severe cryptocaryioniosis were reported in public seawater aquaria, affecting many different teleost species living together in the same container (Nigrelli & Ruggieri, 1966; Colorni, 1985). The disease has also been detected in marine cultures, affecting economically important species as the gilthead seabream and the seabass (Noga, 1995; Diamant et al., 1991). In recent years *C. irritans* is frequently being found in gilthead seabream from cultures of the Spanish Mediterranean coast (Pers. Comm.). These fish usually do not present clinical signs in sea-cages but when kept together in inshore tanks or aquaria, *C. irritans* proliferates and fish becomes heavily infected. This is due to the fact that tomonts cumulate in high number on the bottom of the tanks and theronts can easily re-infect the target hosts usually reared in higher culture density. Water recirculation can increase this effect because theronts are also returned to the system when no disinfection measures are applied to the recirculating water.

*E. leei* is an enteric histozoic myxozoan which parasitizes a wide range of marine fish (Padró et al., 2001; Paperna, 1998; Renaud et al., 2003; Yanagida et al., 2004). This myxozoan has been reported to have caused significant losses in Mediterranean aquaculture, not only affecting gilthead seabream and sharpsnout
seabream cultures but also other sparid species like the black spot seabream (*Pagellus bogaraveo*) and the white seabream (*Diplodus sargus*) (Marino et al., 2004; Golomazou et al., 2006). Currently, *E. leei* is considered a potential threat to the establishment of sharpsnout seabream culture in Italy and Greece where mortalities have reached an overall of 80% (Athanassopoulou et al., 1999).

*C. irritans* and *E. leei* have also affected ornamental fish in public aquaria. Both species have been reported to infect several species in the Valencia public aquarium (Spain) (Ahuir et al., 2004). Padrós et al. (2001) reported *E. leei* in many fish species reared in another large public aquarium in Barcelona, (Spain). In both cases, the most probable origin of the infection seemed to be the contamination by wild fishes of the Mediterranean coast, which were introduced into the aquaria without previous quarantine.

In the parasitoses reported in this study, the parasite also appeared to have been transmitted from infected fish from local natural populations. Thus it seems that both parasite species are abundantly present in Spanish Mediterranean ecosystems, which appear to be a new circumstance as there are not similar reports from recent years. The expansion of these parasites in the Mediterranean could be related to increasing water temperatures due to a global climate change, as previous cases were reported in warmer waters.

The development and diversification of the aquaculture brings along “new” difficulties that need to be resolved. New fish species are forced to live close to other fish species that could share and transmit several pathologies (Di Cave 1998; Woo et al., 2002). This transmission between different fish species is even more feasible possible when one of them has short farming history and its pathogens are not known. Nowadays, the only effective way to control the presence of these two parasites is prophylaxis as no treatment has been found to be effective to eradicate them. Adequate veterinary controls and proper quarantines are indispensable to avoid the wide spreading and contamination of the Mediterranean areas.

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References


