SHORT COMMUNICATION

Occurrence of *Sparicotyle chrysophrii* (Monogenea: Polyopisthocotylea) in gilthead sea bream (*Sparus aurata* L.) from different mariculture systems in Spain

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The exponential increase of gilthead sea bream production in the Mediterranean basin in the last years has been supported by the massive establishment of sea cages, though it has also contributed to the extension and dispersal of parasitic diseases (Sitjà-Bobadilla 2004). The polyopisthocotylean, *Sparicotyle (sin Microcotyle) chrysophrii* is the most threatening ectoparasite for gilthead sea bream cultures. Polyopisthocotyleans have been reported to be responsible for reduced catches in some wild populations, by altering their behaviour and making them more susceptible to predation (Shirakashi, Teruya & Ogawa 2008). They also provoke mortalities in several cultured fish species (Ogawa 2002; Hayward 2005; Montero, Crespo, Padrós, de la Gándara, García & Raga 2004). Some of them are responsible for 22% of total production cost in Australian cultures (see Ernst, Whittington, Corneille & Talbot 2002), and are considered a serious risk for sea-cage aquaculture (Hutson, Ernst & Whittington 2007).

S. chrysophrii has been reported in wild and cultured gilthead sea bream in the Mediterranean basin (Oliver 1984; Fioravanti, Caffara, Florio, Gustinelli & Marcer 2006; Mladineo 2005), sometimes associated to mortalities (Sanz 1992; Alvarez-Pellitero 2004; Vagianou, Athanassopoulou, Ragias, Di Cave, Leontides & Golomazou 2006). It is frequently found in mixed infections with other parasites and secondary bacterial infections (Padrós & Crespo 1995; Cruz e Silva, Freitas & Orge 1997; Caffara, Quaglio, Fioravanti, Gustinelli, Marcer, Moscato & Caggiano 2005). However, there are few long-term studies on this monogenosis and the available data differ greatly depending on the surveyed area. Risk assessment for parasites in aquaculture is of great interest and has to be determined for each particular area and environmental conditions (Hutson *et al*.2007). Furthermore, effective management strategies need to incorporate accurate knowledge of the mode of transmission and the influence of environmental and culture conditions (Altizer, Dobson, Hosseini, Hudson, Pascual & Rohani 2006).

Therefore, the aim of this work was to obtain data on the occurrence of this ectoparasite in Spanish Mediterranean and Atlantic facilities, to design management strategies that could prevent monogenean epizootics. For this purpose, a long-term parasitological survey was conducted in several aquaculture facilities from the Spanish coasts. Two types of samplings were performed. In study A (Table 1), a total of 360 fish from four different Mediterranean ongrowing systems were periodically sampled for 2 years. In all groups, the first sampling was done before fish entered the corresponding facility (thus, it reflects the previous preongrowing situation), and then every three months until fish reached market. The surveyed systems included the intensive indoor experimental tanks of the Instituto de Acuicultura de Torre de la Sal (IATS), and three farms with two different growing systems, sea cages (F-1 and F-2) and intensive earth-ponds (F-3). In study B (Table 2), a total of 300 fish from sea-cage farms F1 and F2 and three additional ones (F-4, F-5 and F-6), and another earth-pond farm (F-7) were occasionally sampled when mortalities or morbidity outbreaks occurred along a wide-ranging period (1999-2007). Most of the surveyed fish farms were located on the Western Mediterranean coast (from Tarragona to Valencia provinces), except F-7, located on the South Atlantic Spanish coast. Figure 1 shows the location of the sampling sites.

All fish were reared under natural temperature and photoperiodic conditions, following the standard procedures of each farm. The mean annual water temperature ranges were 10.5 - 26.3 ºC at IATS facilities, 10 - 28 ºC at F-7, 7 - 30 ºC at F-3, 12.5 - 25.9 ºC at F-6, and 14.2 - 27.6 ºC at the surface in the area of F1, F2, F4 and F5. Figure 2 shows the average monthly temperatures at IATS facilities, which are close to several sampling sites (Fig. 1). IATS seawater supply (37.5‰ salinity) was from a pump on shore, F3 and F7 received water from marsh-land channels and have seasonal salinity oscillations from 34 to 40 ‰. The mean sea water salinity at the area of F-1, F-2, F-4, F-5 and F-6 was

37‰, with slight variations. More details on the sampling conditions for both types of studies are shown in Tables 1 and 2. The monogenean was diagnosed by three methods. Fresh (F) diagnosis: fish were anesthetized with MS-222 (Sigma, Saint Louis, MO) (100 mg/l) and gill scrapings taken from the most external gill arch were examined using light microscopy (LM). The first arch was selected because it is the most parasitized with respect to the remaining arches (Oliver 1984). Stereomicroscope (S) diagnosis: fish were killed by a blow on the head under anaesthesia (MS-222, 100 mg/l), bled to diminish blood content in gills, the whole gills arches excised and examined under the stereomicroscope. In both cases, the type of stages of the monogenean was recorded. Histology (H) diagnosis: after necropsy, gills were fixed in 10 % neutral buffered formalin, embedded in Technovit resin (Kulzer, Heraeus, Germany), 2 µm-sectioned stained with toluidine blue and examined at LM. The anaesthetic procedure was checked previously not to affect monogenean attachment to gill arches. When more than one diagnostic method was applied at a particular sampling, the prevalence of infection was calculated considering any positive fish detected with any method. When using F and H diagnosis, intensity of infection was semiquantitatively evaluated following a conventional scale from 1 to 6+, according to the number of monogeneans per slide (40 observational fields) at 120× (1+ = 1-2 parasites; 2+ =, 3-5; 3+ = 6-8; 4+ = 9-11; 5+ = 12-14; 6+ ≥ 15) . Intensity was quantitatively registered (number of monogenean specimens per fish) with the S method.

Table 1 shows the results of study A. The monogenean was detected only in cage-cultured gilthead sea bream (F1 and F2). It was never found before fish entered the cages, but it appeared as early as the first sampling after introduction (summer period) in both farms. Table 2 shows the infection levels of *S. chrysophrii* during morbidity/mortality outbreaks (study B). Again, the parasite was not detected in earth-pond facilities (F-7).

Prevalence of infection was high in most sea-cages, with presence of adults, juveniles and eggs, even at the end of the winter, regardless of the host weight. Another monogenean, the diplectanid *F. echeneis*, was usually found in the gills of *S. chrysophrii*-parasitized fish, and in some outbreak samplings, epithelocystis organisms and the sanguinicolid *Cardicola aurata* were also present. The concomitant presence of *F. echeneis* and *S. chrysophrii* has previously been reported, even in wild gilthead sea bream (Reversat, Silan & Maillard 1992).

In the present survey, *S. chrysophrii* was not found in indoor facilities, or in the two surveyed earth-ponds facilities, even in fish that had spent more than two years in the ponds. In Italian extensive, earth/sand pond-based farms, the monogenean was neither detected (Fioravanti, Caffara, Florio, Gustinelli & Marcer 2006). However, in other natural environments with earth/sand bottoms and lower water flow than in open sea, like in coastal ponds (Oliver 1984) or lagoons (Vagianou *et al.* 2006), the prevalence of infection in wild and cultured gilthead sea bream reached up to 85 % and 100 %, respectively. The apparent absence of this monogenean in the studied pond-culture facilities could be due to many different factors, such as higher water turbidity, lower water quality, and a higher diurnal and seasonal oscillation of water temperatures due to the low depth of the ponds.

By contrast, *S. chrysophrii* was very prevalent in sea-cages, particularly during outbreak samplings. This monogenean had a moderate prevalence (33.9%) of infection and low abundance (0.46) in Adriatic Sea cages (Mladineo 2005), but infection levels from Mediterranean waters differ depending on the country and the type of facility. Thus, in Italian cages the mean prevalence was 6.1 % (Fioravanti *et al.* 2006), whereas in Greek sea-cages the combined prevalence of *S. chrysophrii* and *F. echeneis* ranged from 61.5 %

to 13.3% (Vagianou *et al.* 2006). Therefore, culture conditions and farm location have a clear effect on the infection levels.

Transmission of some monogeneans exhibits a clear seasonality, with invasion maximised during warmer months (Papoutsoglou, Costello, Stamou & Tziha 1996; Ogawa & Inouye 1997; Mladineo 2005; Rubio-Godoy & Tinsley 2008), and epizootics commonly occur with increasing water temperatures (Ernst *et al*. 2002). Similarly, in the present survey, in both studies (A, B) the highest prevalence and intensity of infection seems to be coincident in most sampling sites with warm water temperatures, which in the studied area are registered in summer and early autumn. The unexpected low values detected in some summer samplings, as in study A in July 2000 in both sea-cages, could be due to formalin, on-site treatments particularly performed by fish farmers in the previous spring. However, from the obtained data it seems that the parasite is capable of resisting winter conditions, as it was detected in F-1 in February and March in studies A and B, respectively. Moreover, the monogenean showed high infection levels and presence of adults and eggs even in the winter of 2005, which was particularly cold (the minimum water temperature was lower than 9 ºC).

In the periodical survey (study A), it was established that fish entering the cages free of the monogenean, can acquire the infection as soon as three months later, regardless of their initial age. In fact, the infection can be experimentally transmitted to naïve gilthead sea bream by contact with *S. chrysophrii* eggs and by cohabitation with parasitized fish in a shorter time, reaching 100 % of prevalence in just five weeks (Sitjà-Bobadilla & Alvarez-Pellitero 2009). Due to the production cycle of gilthead sea bream, which takes about 18-24 months to reach the market size, newly introduced naïve juveniles are held in cages in close vicinity to others holding infected adults. Thus, cage-to-cage transmission could happen in gilthead sea bream stocks, as described for other

fish-monogenean models (Chambers & Ernst 2005). However, no field experimental data are available to confirm it.

From the information obtained in this and previous parasitological studies, we identify *S. chrysophrii* as a real risk for gilthead sea bream sea-cage cultures. It is clear that sea-cages provide the ideal conditions for the continuous exploitation of the host by the parasite, as it can survive winter conditions in the cages and spreads easily to new stocked animals. Net biofouling not only provides a suitable place for the entanglement of monogenean eggs, but also prevents a high water flow, which is essential for animals with diminished gill function. Thus, we strongly recommend synchronization of bath treatments, and net cleaning with the introduction of new animals in the facilities. The knowledge of the minimum distance between neighbouring sea-cages that would avoid parasite dispersal would help to the management of this monogenosis.

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242 **Figure legends**

243 Figure 1. Approximate location of sampling sites (Instituto de Acuicultura de Torre de la
244 Sal = IATS, and farms = F) of *Sparus aurata* on the Spanish coasts which were
245 checked for *Sparicotyle chrysophrii* infection.

246 Figure 2. Monthly water temperatures at the indoor facilities of the Instituto de
247 Acuicultura de Torre de la Sal (Castellón province).

Table 1 Study A. Sampling conditions and infection levels in gilthead sea bream periodical samplings of indoor facilities (IATS), earth ponds (F-3) and sea cages (F-1, F-2). P = prevalence, MI = mean intensity.

Samplings					*Sparicotyle chrysophrii*		
Farm (province)	Time[a]	Fish weight[a] (g) (mean ± SD)	Type of diagnosis[c]	n	P (%)	MI	Stages
IATS (Castellón)	Mar 99 - Jul 00	20.5 ± 3.7 – 373.3 ± 61.9	F	150	0	-	-
F-1 (Castellón)	Mar 99[b]	21.3 ± 4.7	F	10	0	-	-
	Jul 99	92.3 ± 12.9	F	10	50	1.6+	J, A
	Oct 99	222.9 ± 35.1	F	10	10	1+	A, E
	Feb 00	313.5 ± 53.4	F	10	30	1+	A
	Jul 00	339.8 ± 50.2	F	10	0	-	-
F-2 (Tarragona)	Apr 99[b]	8.5 ± 2.1	F	10	0	-	-
	Jul 99	31.8 ± 8.9	F	10	40	1.5+	J, A, E
	Oct 99	110.3 ± 19.5	F	10	0	-	-
	Jan 00	142.4 ± 31.2	F	10	30	1.7+	A, E
	Apr 00	175.7 ± 38.6	F	10	50	1.2+	A, E
	Jul 00	281.3 ± 48.3	F	10	10	1+	J
F-3 (Tarragona)	May 99 - Oct 00	2.9 ± 0.6 – 414.1 ± 76.5	F	90	0	-	-

[a] When the parasite was not detected in a facility, sampling, time period and fish weight at the initial and final samplings are indicated, instead of the data at each sampling date. [b] First sampling before entering the cages. [c] The parasite was diagnosed with the fresh diagnosis (F) method, and the stages determined as J = juveniles, A = adults, E = eggs.

Table 2 Study B. Sampling conditions and infection levels in gilthead sea bream in outbreak samplings of sea cages (F-1--6) and earth ponds (F-7). P = prevalence; MI = mean intensity.

Samplings						*Sparicotyle chrysophrii*		
Farm (province)	Time	Months in cages	Weight (g) (mean ± SD)	Type of diagnosis[a]	n	P (%)	MI	Stages
F-1	Apr 99	5	123.4±36.7	F/H	10	20	1.5+	J, A, E
(Castellón)	Mar 00	3	17.2±5.9	F/H	10	100	3.8+	J, A, E
	May 00	6	67.9±11.7	F/H	10	0	-	-
	Jun 03	8	300.1±48.3	S	15	93.3	8.6	J, A, E
	Oct 04	7	76.8±15.4	S	10	100	13.6	A, E
	Mar 05	3	32.1±5.2	F/H	20	60	2.2+	A, E
F-2 (Tarragona)	Jul 99	4	127.6±49.6	F/H	8	25	1+	A, E
F-4	May 03	13	200.1±20.3	S	8	100	6.9	J, A, E
(Castellón)	Jun 03	7.5	47.6±6.9	S	13	100	70.6	J, A, E
	Nov 04	3.5	31.7±4.3	F/H	20	0	0	-
F-5	Jun 04	8	60.3±8.9	S	20	100	12	A, J
(Castellón)	Oct 04	9	90.1±13.7	S	20	100	52.4	J, A, E
F-6 (Valencia)	Nov 07	7	104.2±15.3	F/H	18	11.1	1+	A, J
F-7	Jun 06	1	15.4±3.2	F/H	20	0	-	-
(Cádiz)	Jun 06	3	55.7±14.8	F/H	20	0	-	-
	Oct 06	6	205.9±44.9	F/H	20	0	-	-
	Oct 06	> 24	158.1±51.9	F/H	20	0	-	-
	Sep 07	5	77.7±20.9	F/H	17	0	-	-
	Sep 07	>16	238.2±85.8	F/H	21	0	-	-

[a] The monogenean was diagnosed by the fresh (F), histology (H) or stereomicroscope (S) diagnosis methods, and the stages determined as J = juveniles, A = adults, E = eggs.

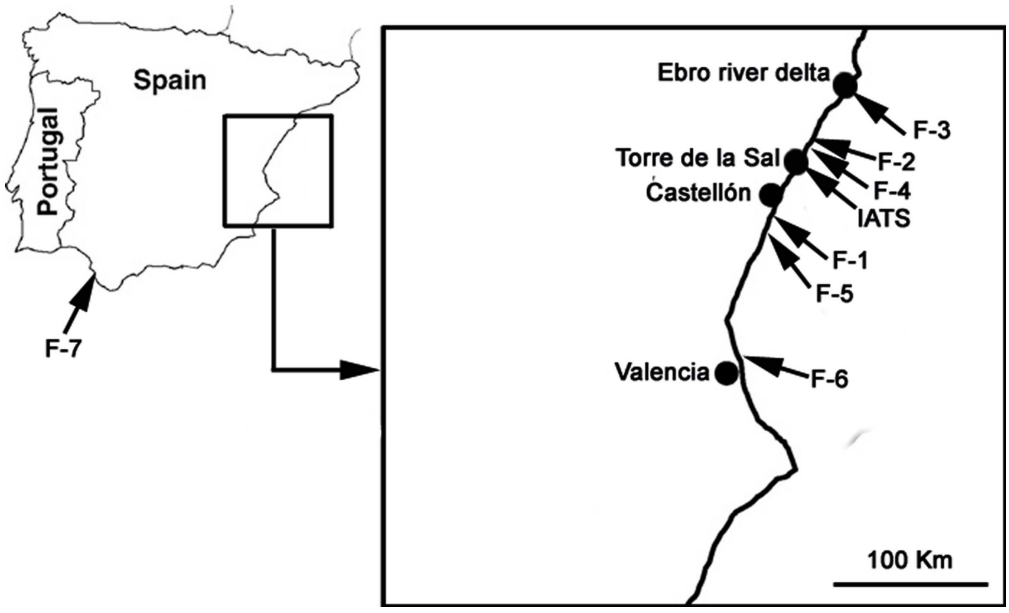

Fig.1
99x59mm (600 x 600 DPI)

Fig. 2 Sitjà-Bobadilla et al.