Mating behaviour and gamete release in gilthead seabream (Sparus aurata, Linnaeus 1758) held in captivity

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Abstract

The present study aimed to describe the reproductive behaviour of gilthead seabream (Sparus aurata) in captivity. Twenty-four mature gilthead seabream, divided in two tanks, were utilized for the present study. Reproductive behaviour was recorded using submersibles cameras. A total of 67 spawning events were analysed. The mean duration time that gilthead seabream spent spawning was 54 ± 4 min/day, during which mean number of individual spawning events was 5.6 ± 0.2. The mean volume of eggs produced by both broodstocks was 405 ± 13.4 mL with a fertilization rate of 91.6 ± 0.4%. The reproductive behaviour began with a schooling behaviour and then forming light aggregations. From an aggregation or an encounter while swimming freely a female initiated a spawning rush followed by one or more males to gametes liberation. The spawning rush was brief, 1.6 ± 0.5 sec, over an approximately 1.7 ± 0.2 m distance from the tank bottom to the water surface. Pair spawning, between a single female and male, was the most common (71.6%). Group spawning was less common and involved a single female spawning with two males (22.5%) or three males (4.9%). Spawning rushes involving more than one female were not observed. Gilthead seabream in the present study presented a tendency to pair spawn and eggs collected as a “spawn” were actually the sum of many separate spawning events over a short time period. This is the first description of gilthead seabream spawning and the findings help to understand microsatellite based observations of spawning kinetics.

Additional key words: mate selection; pair group mass spawning; rush; aggregation

Citation: Ibarra-Zatarain, Z.; Duncan, N. (2015). Mating behaviour and gamete release in gilthead seabream (Sparus aurata, Linnaeus 1758) held in captivity. Spanish Journal of Agricultural Research, Volume 13, Issue 1, e04-001, 11 pages. http://dx.doi.org/10.5424/sjar/2015131-6750.

Received: 27 Aug 2014. Accepted: 11 Feb 2015 http://dx.doi.org/10.5424/sjar/2015131-6750

This work has one supplementary video that does not appear in the printed article but that accompanies the paper online.

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Funding: This study was funded by the EU-research for SMEs project REPROSEL; Grant Agreement nº FP7-SME-2010-1-262523-REPROSEL coordinated by Herve Chavanne; the INIA-FEDER Project RTA2011-00050 coordinated by ND; and the PhD grant awarded to ZIZ by CONACYT, Mexico.

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Gilthead seabream (Sparus aurata), from the Sparidae family, is one of the most extensively farmed fish species in the Mediterranean region. During the last two decades, many studies have described aspects of the biology of the species, including reproduction and genetics (Holland et al., 1998; Almansa et al., 1999; Meiri et al., 2004; Rossi et al., 2006; Arabaci et al., 2010; Mylonas et al., 2011). Gilthead seabream is a protandrous hermaphrodite species with an asynchronous ovarian development (Zohar et al., 1995). Broodstock held in captivity under natural conditions typically start vitellogenesis in September-November, spawning begins during December-January and lasts for 3-5 months with daily spawning, leading to an annual fecundity of 2,000,000 eggs/kg (diameter<1 mm) with a fertilization rate of 80-85% (Barbaro et al., 1997; Arabaci et al., 2010; Mylonas et al., 2011). However, the reproductive behaviour of gilthead seabream has not been reported, despite an increasing need to understand the factors that influence a breeders participation in spawning in order to control the families produced from a broodstock (Gorshkov et al., 1997; Brown et al., 2005; Porta et al., 2009; Chavanne et al., 2012).

Reproductive behaviour has been described in some species of the family Sparidae, kept in captivity, including silver seabream (Chrysophrys auratus) (Smith, 1986; Mylonas et al., 2011), santer seabream (Cheime-
ment programmes. The aim of the present study was to investigate and describe the particularities of reproductive behaviour of the gilthead seabream in rearing conditions.

Material and methods

Ethic statement

All the experimentation on fish that formed part of this study were in agreement with the Spanish and European regulations on animal welfare (Federation of Laboratory Animal Science Associations, FELASA) and approved by the Animal Ethics Committee of IRTA.

Fish maintenance

Twenty four mature gilthead seabream (Sparus aurata) with a mean weight of 2.59 ± 0.15 kg and a length of 49 ± 4 cm were used for this study. Fish were pit-tagged for identification and divided among two 16.2 m$^3$ rectangular (6 × 3 × 0.9 m) fibreglass tanks (identified ahead as C1 and C2). Sex ratio per tank was 7 females and 5 males; a ratio biased to females is commonly used in the industry. Females were larger and older (mean weight: 2.91 ± 0.12 kg) than males (mean weight: 2.27 ± 0.19 kg), and this morphological difference was established as the main criteria to distinguish males from females in the video recordings.

Tanks were located outside in a greenhouse structure covered with shade netting. Photoperiod was adjusted to follow the natural seasonal cycle by using two halogen white lights installed inside of each tank. Lights turned on-off in tanks with a photocell sensor. Water temperature and oxygen were maintained between 18-19°C and 5-6 mg/L, respectively. Fish were fed, ad-libitum, daily in the mornings (between 09:00-10:00 hours) with a commercial extruded balanced diet (Vitalis CAL-9, Skretting, Burgos, Spain).

Video and observations of the reproductive behaviour

Fish behaviour was recorded with four submersible black and white cameras (F60B/NIR580-50G model, Korea Technology Co. Ltd, supplied by Praentesis S.L., Barcelona) connected to a recorder (DVR- 0404HB model, Dahua Technology Co. Ltd, supplied by Praentesis S.L., Barcelona). Cameras were installed in each tank 5 cm under the water surface and adjusted to
achieve a field of vision that covered more than 95% of the area and water column of the tanks.

The video recording was completed on different dates for both tanks as only one video recording system was available. Tank C1 behaviour was recorded from 10th to 24th January and from the 1st to the 4th February 2012; subsequently, tank C2 was recorded from the 5th to 14th February and from 30th of May to 7th of June 2012. The video recording program was daily starting at 08:00 until 13:00 hours in both tanks. This schedule was determined in relation to egg collection, generally collectors were observed to be empty at 08:00 hours and after collection at 13:00 hours no more eggs were collected until the following day after 08:00 hours.

Focal animal observations of spawning behaviour and behaviour in general were made from the recorded videos following recommendations published by Altman (1974). A total of 67 spawning events were analysed. In tank C1, spawning observations corresponded to days 12th, 13th, 16th and 18th January and 1st-2nd February, whilst in tank C2, observations corresponded to days 05th, 09th-12th February and 30th May. The following types of behaviours and observations were described from the videos: i) pre-spawning interactions between individuals or in a group, ii) the behaviour directly associated with gamete liberation, iii) fish aggregation patterns and duration, iv) number of fish participating in each spawn (pair or group spawning) and the sex proportion per spawn, v) the frequency, duration and position of fish in tank when spawning and vi) estimation of the average distance (estimated from known distances between reference points in the tank) of the spawning rush. These parameters were selected in accordance to previous work realized on Sparidae species (Smith, 1986; Buxton & Garratt, 1990; Garratt, 1991; Leu, 1994; Mylonas et al., 2011) and in particular terminology defined by Domeier & Colin (1997) was used to describe behaviours and actions. These included the following definitions of types of spawning from Domeier & Colin (1997) “Pair spawning: spawning by a single male and single female. Group spawning: rush consisting of more than two fish, often many individuals. The group usually consists of a single female and multiple males. Mass spawning: a form of group spawning that consists of the great majority to all of an aggregation spawning simultaneously, as a single unit”.

Eggs collection and evaluation

Egg collection was daily between 11:30 and 12:00 hours from both tanks. A 2-L measuring cylinder was used to measure the total volume of spawned eggs and the fertilization rate was determined by counting fertilized and unfertilized eggs from a sample of 50 eggs. Fertilized eggs were identified by observing cellular divisions, while unfertilized eggs did not present any cellular divisions. Likewise, the developmental stage of the embryonic phase of eggs was analyzed and established with accordance to Kamaci et al. (2005), in order to corroborate the estimates of spawning time obtained from videos with the developmental stage of eggs.

Statistics

All data were expressed in mean ± S.E.M. Student’s t-test was performed to compare different behavioural patterns between the two broodstocks (tank C1 and C2), such as the total number of aggregations prior a spawning, spawning duration, frequency of spawns per day, the distance displaced to spawn and the sex proportion per spawn. Pearson correlation test was performed between the number of daily events of gamete release and the volume of eggs collected. All the statistical analyses were conducted using SPSS software (Chicago, IL, USA) and a significant difference was considered when p < 0.05.

Results

Observations and description of the sea bream reproductive behaviour

Based on the video-observations, the gilthead seabream reproductive behaviour was divided into two phases: the pre-spawning and the spawning behaviour. It was noted that seabream in the present study had a tendency to spawn daily in both tanks with close to all eggs being spawned between 08:00 and 11:00 hours. However, a small number of spawns were outside of these hours.

Pre-spawning behaviour

— Resting behaviour: Resting behaviour was observed when lights in both tanks were turned on (on average activated by photocell sensor at 08:30 hours). This behaviour was characterized with fish totally dispersed, without interactions and disaggregated around the tanks, and fish swam alone or in small groups around the tank without any specific direction or preference (Fig. 1, Table 1).
— Schooling behaviour. On average 42 ± 8 min after
the lights were turned on, fish behaviour changed and
fish started to form groups and swim together follow-
ing one behind another from one side of the tank to the
other. However, fish did not present any specific direc-
tion or preference, but always were swimming near the
bottom of the tank. It was also observed that some fish
maintained a reduced distance in relation to other fish
and this included some fish touching or sneaking after
each other (Fig. 1, Table 1). This behaviour pattern was
observed daily in both tanks for approximately 10 min
and prior to the aggregation behaviour. However, no
particular leading fish or inter-individual dominance
between fish could be observed amongst the individu-
als of both tanks.

Spawning behaviour

— Aggregations and courtship behaviour. Aggrega-
tions and courtship behaviour commenced when the
whole group of breeders started to form aggregations
near the bottom of the tanks (Fig. 1, Table 1, Suppl.
Video S1), being comparable to a “loose ball” and occa-
sionally aggregations became tighter as the fish swam
closer together; nonetheless, in the majority of the
observations a “tight ball” of fish was not formed. Also,
during this stage, males were observed to become
slightly darker and occasionally males were rubbing
and nudging (Fig. 1, Table 1) some females close to
the genital pore. The change in colour of males in ad-
tion to differences in size between males and females
was also used to identify males. Territorial dominance
or aggression amongst fish of the same or different sex
was not observed.

In parallel to the aggregation behaviour, fish (males
and females) initiated the courtship behaviour, which
was mostly brief, and started when one of the females
increased swimming speed at the bottom of the tank
and slightly separate from the rest of the group, al-
though on repeated occasions this was punctuated by
immobile periods of the female in mid-water column
and periods of circling aggregation behaviour as de-
scribed above. After 10-15 sec of this behaviour, the
female with one or more males was observed to dra-
matically increase swimming speed to initiate the
spawning rush (Fig. 1, Table 1). Aggregation and court-
ship behaviour ranged from 5 to 70 min to average
21 ± 4 min in both tanks.

— Spawning rush. The spawning rush was observed
to follow when either i) the female started a circling
behaviour followed by the male(s), which again pro-
duced the “loose ball” aggregation behaviour described
previously, but the female would then exit from the
group at speed or ii) after an apparently coincidental
brief encounter with a male, the female dramatically
increased swimming speed. This dramatic increase in
swimming speed by the female was in all cases from
low in the water column close to the bottom in a diago-
nal line towards the water surface. The female swam
rapidly away from other fish followed by one or more
males (Figs. 1 and 2; Table 1, Suppl. Video S1). Gamete
release, egg and sperm, were synchronous at speed dur-
ning the rush and in the top half of the water column.

The rush lasted in average 1.6 ± 0.5 sec in both tanks
(Fig. 1). During this phase, the female was mostly

![Figure 1](Image)

Figure 1. Average time periods of the different behavioural patterns observed in the gilthead
seabream during the pre-spawning and the spawning events.
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Disaggregation. The spawning behaviour (from the formation of the aggregation to the end of the spawning rush) ended when the group of fish disaggregated and returned to a resting behaviour, with fish dispersed, swimming in all directions and biting the floor as if they were looking for food (Fig. 1). In addition all the fish in the group presented a similar colour and no dark males were observed, which also appeared to indicate the end of the spawning behaviour.

Spawning pattern in both broodstocks

— Aggregations and courtship behaviour. From the 67 spawning events observed, 35 corresponded to the

| Behaviour                | Description                                                                 |
|--------------------------|-----------------------------------------------------------------------------|
| Resting                  | Fish are disaggregated around the tank                                       |
| Schooling                | Breeders form groups, start to swim together with uniform movements          |
| Rub-Nudge                | Male approach gravid female and makes physical contact, with mouth, in the lower abdomen near the genital pore |
| Aggregation-courtship    | Males swim near the females forming like a tight ball and made several contacts, this pattern commonly preceded the spawning rush of fish |
| Spawning rush            | Female and a male separates from group and swim rapidly in a straight line while close together, male directly behind (at times touching) the female and oriented in the same manner. Rushes vary in direction from diagonally vertical (most common) to horizontal (rare); rush ends with synchronized gamete liberation, after which the fish separated |

Figure 2. Video captures of the gilthead seabream spawning rush. P shows two examples of a pair spawning with the female followed by the male; G shows two examples of group spawning, the upper photo shows a female followed by two males and the lower photo a female followed by three males.
bers or frequency of spawns per day recorded were 5.83 ± 0.21 in tank C1 and 5.33 ± 0.32 in tank C2 (Table 2); and after completing the spawning rush breeders, from both tanks, returned back to the group.

— Distance of spawning rush and preferred area to spawn. The approximate distance displaced by broodstock in tank C1 (1.8 ± 0.2 m) to liberate the gametes during the spawning rush was no different from broodstock held in tank C2 (1.6 ± 0.3 m) (Table 2). Also, on the 67 recorded spawning events it was observed that seabream spawned 39.8% in the water inlet area of the tanks, 37.4% in the middle part and, finally, 22.7% spawns occurred in the water outlet area of the tanks. It was also observed that seabream spawned in the majority of occasions near the water surface.

— Eggs volume, fertilization rate and developmental stage. Regular daily spawning began in both tanks in early January and spawning finished in June. The peak period of spawning in both tanks extended late January to mid-April and during the period 24 Jan to 15 April the mean volume of daily floating eggs were 434 ± 193 mL from tank C1 and 273 ± 155 mL from tank C2. On the days selected to analyse the spawning

Table 2. Means values of different spawning patterns observed in the gilthead seabream.

| Spawning parameter                  | Tank C1       | Tank C2       |
|-------------------------------------|---------------|---------------|
| Number of spawn with aggregations   | 25            | 13            |
| Number of spawn without aggregations| 10            | 19            |
| Number of spawn with courtships     | 20            | 14            |
| Mean distance displaced (m) per rush| 1.8 ± 0.2     | 1.6 ± 0.3     |
| Mean eggs volume (mL) spawned       | 343 ± 12      | 467 ± 15      |
| Mean fertilisation rate (%)         | 95 ± 0.2      | 88 ± 0.5      |

Figure 3. Sex proportion per spawning in the gilthead seabream (Sparus aurata) held in captivity.

Spanish Journal of Agricultural Research  March 2015 • Volume 13 • Issue 1 • e04-001
behaviour the breeders in tank C1 spawned a daily mean of 343 ± 12 mL and in tank C2 spawned 467 ± 15 mL (Table 2). No correlation was found between the number of spawning events per day and the volume of eggs collected ($R^2 = 0.2323$, $p > 0.221$). Eggs collected from tank C1 presented a fertilization rate of 95 ± 0.2%, while in tank C2 the mean fertilization rate was 88 ± 0.5% (Table 2). The embryonic phase of development of the collected eggs were mainly between 2 and 32 cell division (phases 1A to 1E as defined by Kamaci et al., 2005); nonetheless, on occasions it was observed some eggs to be in morula or gastrula phase (1F and 1G) and these developmental phases corresponded to the timing of the observed spawning events.

**Discussion**

The present study described, for the first time, the reproductive behaviour of gilthead seabream (*Sparus aurata*) held in captivity. The reproductive behaviour was similar to that described for other Sparidae species (Smith, 1986; Buxton, 1990; Buxton & Garratt, 1990; Garratt, 1991; Leu, 1994; Mylonas et al., 2011). Gilthead seabream were observed to form defined aggregations prior to the spawning event and females were observed to make a spawning rush with one or more males that finished with gamete liberation.

In accordance with Domeier & Colin (1997) the aggregation behaviour performed by fish was defined as a group of conspecific fish that gathered for the purpose of spawning, with fish densities or numbers significantly higher than those found in the area during the non reproductive period. In the present study, gilthead seabream aggregations were well defined, included the participation of all the stock and were clearly associated with spawning. The courtship behaviour of gilthead seabream was mostly brief and characterized by rapid forward swimming by females followed by one or more males. In addition, males displayed two characteristics: a colour change to become slightly darker and nudging and rubbing the female’s bellies close to the oviduct. The formation of aggregations and the courtship (changes in swimming speed, colour changes and nudging) appeared to offer the opportunity for mate selection and brought all the available individuals together for mate selection. Dichromatism (ability to take on one of two different colours patterns separately) was suggested to be a motivational factor for females to select males with better physical condition and social status (Kodric-Brown, 1998; Okumura et al., 2002; Kline et al., 2011). The action of rubbing and nudging was hypothesized to help males to perceive female pheromones, trigger the ovulation and induce the oocytes liberation (Bond, 1996; Domeier & Colin, 1997; Heyman et al., 2005; Stacey & Sorensen, 2008). In the present study, obvious behaviours associated with gaining dominance were not observed between males or males and females. However, a passive process of selection between males and females can be suggested as both observations of behavioural and morphological aspects appeared to offer opportunities for females to accept or reject advances from males. These indications that presented opportunities consisted of: a) spawning was often in a pair indicating the pair could select each other, b) aggregations brought all the fish together for close contact to aid selection and spawning was often soon after an aggregation, c) males followed females perhaps seeking selection, d) males nudged females to possibly stimulate selection, e) females were observed to swim away from advances from males and f) males changed colour changing appearance to perhaps aid selection by the female. Aggregations and/or courtship behaviours similar to the present study have been described in other species of Sparidae including silver seabream (*Chrysophrys auratus*) (Smith, 1986; Mylonas et al., 2011), santer seabream (*Cheimerius nufar*) (Buxton & Garratt, 1990; Garratt, 1991), roman seabream (*Chrysolebphus laticeps*) (Buxton, 1990), silver bream (*Rhabdosargus sarba*) (Leu, 1994) and southern black bream (*Acanthopagrus butcheri*) (Mylonas et al., 2011) and non-Sparidae such as the spotted sand bass (*Paralabrax maculatofasciatus*) (Miller & Allen, 2006), yellowtail amberjack (*Seriola lalandi*) (Moran et al., 2007), dusky grouper (*Epinephelus marginatus*) (Zabala et al., 1997), cubera snapper (*Lutjanus cyanopterus*) (Heyman et al., 2005) and white seabass (*Atractoscion nobilis*) (Aalbers & Drawbridge, 2008).

However, in the present study, aggregations were not always observed immediately prior to gilthead seabream spawning and no inter-individual dominance were observed. Liberation of gametes was observed both in gilthead seabream coming from an aggregation (with or without courtship) and fish that had not participated in aggregation (or courtship) behaviour immediately prior to spawning. However, the importance of these social interactions (aggregations and courtship) during the spawning period should not be lessened by these observations. Gilthead seabream spawning success was low when held in pairs (Gorshkov et al., 1997; N. Duncan, pers. obs.) or groups of 15 females with a single male (Gorshkov et al., 1997). Holding gilthead seabream in pairs or 15 females with a single male would be too few fish or the wrong sex ratios to enable the social interactions (aggregations and courtship) observed in the present study and this may explain the poor spawning success observed in
gilthead seabream held in pairs or small groups (Gorshkov et al., 1997; Duncan et al., 2013). Various authors have suggested large groups of breeders were required for successful spawning of gilthead seabream (Gorshkov et al., 1997; Duncan et al., 2013) and Sparidae in general (Pankhurst, 1998; Mylonas et al., 2011).

In the present study, gilthead seabream made a spawning rush with a preference to rush and spawn as a pair and 71.6% of total spawns were observed to be between a single female and male. However, gilthead seabream were also observed to group spawn when one female spawned with several males: two (22.5%) or three males (4.9%). Species from the Sparidae family all presented a spawning rush and different species presented pair or group or both types of spawning. The silver seabream (Smith, 1986; Mylonas et al., 2011) and santer seabream (Buxton & Garratt, 1990; Garratt, 1991), like the gilthead seabream presented both pair and group spawning. However, silver seabream were predominantly group spawners with one female being followed by many males (Smith, 1986; Mylonas et al., 2011), but pair spawning was observed on one occasion (Smith, 1986). Santer seabream pair spawned (Buxton & Garratt, 1990; Garratt, 1991) and the dominant male was aggressive towards other males, however, on occasions a “streaker” or “sneaker” male was observed to successfully participate in spawns by keeping to the opposite side of the female to the dominate male (Garratt, 1991). In the present study, no evidence of sneakermale was observed in gilthead seabream, although, when group spawning was observed there was always a lead male closest to the female followed by a second and less frequently a third male. The roman seabream (Buxton, 1990) and silver bream (Leu, 1994) were only observed to pair spawn and the southern black bream was only observed to group spawn (Mylonas et al., 2011). To date no Sparidae species has been observed to mass spawn and the observed pair and/or group spawning preceded by social interactions related to mate selection were characteristic of gilthead seabream and other Sparidae species.

Domeier & Colin (1997) defined a mass spawning as “a form of group spawning that consists of the great majority to all of an aggregation spawning simultaneously, as a single unit”. Studies on parental assignment of progeny (Brown et al., 2005; Porta et al., 2009; Chavanne et al., 2012) and this variation or dominance by certain fish was particularly clear amongst male breeders (Brown et al., 2005). A similar situation was observed in the parental assignment of male cod breeders to progeny (Bekkevold et al., 2002) and this coupled with observations of cod reproductive behaviour (Brawn, 1961; Hutchings et al., 1999) suggested that cod males had reproductive hierarchies that explained the dominance of progeny by certain males (Bekkevold et al., 2002). A similar coupling of the present study on gilthead seabream spawning behaviour with studies on parental assignment of gilthead seabream progeny (Brown et al., 2005; Porta et al., 2009; Chavanne et al., 2012) also suggested the hypothesis that gilthead seabream had reproductive hierarchies that resulted in the dominance of progeny by certain breeders particularly amongst males. Chavanne et al. (2012) concluded that further research was required to understand the spawning kinetics of gilthead seabream. The present study, highlights that such studies need to also focus on spawning behaviour to understand why certain fish dominate spawning in relation to the spawning environment considering both physical (tank design, size) and social (characteristics of individuals, sex
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ratios, density) aspects. This, the first description of gilthead seabream spawning behaviour provides an important bases for these studies and for the first time researchers and broodstock managers can have a clear idea of the spawning behaviour when considering physical and social manipulations to increase parental contribution for breeding programs.

In the present study, the spawning activity took place midmorning, which was actually initiated 42 ± 8 min after the lights switched on and can be considered similar to previous studies that established that gilthead seabream and others sparid fish such as silver seabream (Sparus sarba), Pacific seabream (Acanthopagrus pasicificus), yellowfin bream (Acanthopagrus australis), red seabream (Pagrus major) and black bream (Acanthopagrus butcheri) tend to spawn at sunset or early in the morning (Pollock, 1982; Matsuyama et al., 1988; Mi-helakakis & Kitajima, 1995; Haddy & Pankhurst, 1998; Meseguer et al., 2008; Sheaves & Molony, 2013). In the present study, spawning was successfully and regularly obtained and presented a prolonged spawning season (up to 5 months). Spawning was close to every day in both tanks. These observations were characteristic of this species, and in accordance with Zohar et al. (1995), Barbaro et al. (1997) and Arabaci et al. (2010).

The present study demonstrated that gilthead seabream spawning behaviour was similar to other sparids. In most occasions, spawns were observed to initiate in the morning hours and presented the characteristic to be associated with aggregation behaviour, followed by the spawning rush performed by a single female pursued by a male or, less common, by two or three males. Aggregation and courtship behaviour appeared to be an essential part of the spawning behaviour probably related to mate selection, highlighting the need to have a group of breeders and not single pairs. These findings described for the first time the characteristics of gilthead seabream reproductive behaviour and that many spawning events during a short space of time were involved in the production of a “spawn”. Altogether the study provided valuable information that may explain the uneven participation of breeders in studies that determined paternity of progeny with microsatellites and provides a solid basis for future work to increase parental contributions to breeding programs.

Acknowledgements

We are grateful for the assistance given by Feliu Ferre, Josep Lluís Celades and Esteban Hernandez and other technical staff from IRTA, Sant Carles de la Ràpita, for maintaining the fish and assistance with the installation of equipment.

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