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Brood stock formation of the hermaphrodite finfish species *Pagellus erythrinus* (common Pandora) from fish reared in captivity

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Abstract

Formation of brood stock is considered to be one of the most important operations in order to acquire eggs and fry from any promising candidate finfish species for aquaculture production. The sex reversal observed in hermaphrodite species adds to confusion and creates additional complications in forming a brood stock. The present study describes the efforts and the results of the brood stock formation of the hermaphrodite finfish species Pagellus erythrinus (common Pandora) from individuals aged between 4 and 5 years (TL>300mm) reared in floating cages. Six groups were formed (50 fish/group) in all of which females were present comprising 20 to 40% of the population. The presence of females was in contrast to the literature, which reported that sex reversal of the common Pandora is complete in naturally occurring populations with the absence of females in sizes of a total length greater than 220mm, indicating that in captivity sex reversal is not complete for this species. Four of the groups formed spawned under natural environmental conditions without hormonal treatment and the other two groups were administered a different dosage (250 and 500 IU/kg) of Human Chorionic Gonadotropin (HCG) to induce spawning. The reproductive period started in the middle of May and ended at the beginning of July and spontaneous spawning occurred in all groups. Egg release lasted for a mean period of one month for the groups that spawned without hormonal treatment with no significant difference in the number of viable eggs between groups. The groups that spawned under hormonal treatment released eggs for a period of six and seven days, for the group that spawned under the high and low hormonal treatment, respectively, with no significant difference in the number of viable eggs between them. The hormonal induced spawning resulted in egg release within a short period of time ideal for a hatchery. However, the number of viable eggs produced was significantly lower compared to the number of viable eggs produced from groups that spawned without hormonal treatment.

Keywords: Hermaphrodite, brood stock, *Pagellus erythrinus*, common Pandora.

Introduction

Mediterranean finfish mariculture is at present dominated by the intensive and highly technified production of gilthead sea bream *(Sparus aurata)* and European sea bass *Dicentrarchus labrax.* One of the main strategies to ensure the future expansion of Mediterranean aquaculture is species' diversification (HOUGH, 1996; STEPHANIS, 1996; BASURCO & ABELLAN, 1999), targeting species of high market value that are easy to domesticate and rear (STEPHANIS & DIVANACH, 1993). Diversification poses a great challenge for further aquaculture development and focuses on a few species, most of which belong to the family Sparidae playing an important role in world production (BASURCO & ABELLAN, 1999). The potential of Sparids for use in aquaculture, relies on the high degree of development achieved with some species of this family such as the red porgy *(Pagrus pagrus)* and the gilthead sea bream *(Sparus aurata)* and in particular to new species with similar biological characteristics (HUSSAIN *et al.*, 1981; GIRIN, 1982; CEJAS *et al.*, 1993; FERNANDEZ-PALACIOS *et al.*, 1994; MENDEZ *et al.*, 1995).

Economically viable aquaculture depends heavily upon a reliable supply of fertile eggs and juvenile fish, which can be produced only by the formation and maintenance of cultured brood stock. The common Pandora *(Pagellus erythrinus)* (Linnaeus, 1758) is a demersal fish belonging to the Sparidae family, extending geographically from the Black sea and the Mediterranean, along the west coast of Europe and Africa, to Norway and Angola. It can be found in depths down to 300m but is most common at 20 - 100m (BAUCHOT & HUREAU, 1986; MYTILINEOU, 1989; SANTOS *et al.*, 1995). This species exhibits protogynous hermaphroditism (ANDALORO & GIARRITTA, 1985; GIRARDIN & QUIGNARD, 1985; FISCHER *et al.*, 1987; PAPACONSTANTINOU *et al.*, 1988; LIVADAS, 1989; PAJUELO & LORENZO, 1998), and together with gilthead sea bream *(Sparus aurata)* and red porgy *(Pagrus Pagrus)* comprise three of the most promising species of the Sparidae family for large-scale aquaculture in the Mediterranean region.

Larraneta (1964) reports that the ovarian region of the common Pandora persists along the dorsoventral surface of the testes, in the form of degenerated tissue in all males when the testicular region acquires the characteristic of the testes. Hermaphroditism is a phenomenon commonly observed in fish occurring with a complex form in the Sparidae family (Atz, 1964). Taking into consideration that at present the majority of the Mediterranean species candidates for aquaculture diversification belong to this family (BASURCO & ABELLAN, 1999), the necessity of forming a successful brood stock is of major importance. In the case of a hermaphrodite species the knowledge of the species' biology, including the type of hermaphroditism, age of gonad maturation, initial sex expressed and the exact time of sex reversal (REINBOTH, 1962; ATZ, 1964), are of vital importance. In the family Sparidae, gonads appear as a two-sexed organ in which the male and female tissues coexist in separation. Furthermore, each gonad section develops and functions successively, with either the male or the female side functioning initially. The 'complete' hermaphroditism (REINBOTH, 1962; ATZ, 1964) results in sex reversal observed at a certain age with the degeneration and atrophy of the currently functioning tissue and the consecutive initiation of the tissue in cessation. The individual will then develop and function as a member of the opposite sex until the end of its life. The phenomenon of 'complete' hermaphroditism in not universal for all the individuals of a population and instead a number of individuals will constitute 'incomplete' hermaphrodites which will not undergo sex reversal. In protogynous hermaphrodite species for example a number of individuals will develop testicular tissue and undergo ovarian degeneration before the onset of sexual maturity, thus functioning as males throughout their life omitting the female phase (REINBOTH, 1962; ATZ, 1964). In the case of the red porgy (Pagrus pagrus) two kinds of males coexist in a given population; the 'secondary males' comprise the previously female individuals that have undergone sex reversal (complete hermaphroditism), and the 'primary males' comprising of individuals in which before the onset of sexual maturity, the ovaries never became functional and degenerate developing only testicular tissue (incomplete hermaphroditism) (KOKOKYRIS *et al.*, 1999). The combination of 'complete' and 'incomplete' hermaphrodites creates complex population structures within a population, further complicating the formation of a successful brood stock of a hermaphrodite species.

In the case of the gilthead sea bream, a protandrous species, the addition of young males in the brood stock, during the period between the end of the reproductive period and the initiation of the new one, is vital since the number of older males that will undergo sex reversal will be increased. On the contrary, with the addition of females, older males will not undergo sex reversal and the quantity of sperm produced from the males will not be adequate to fertilise the entire number of eggs produced (ZOHAR *et al.*, 1995). It is thus important to properly select the right individuals for a successful brood stock formation and periodically manage the population by replenishment or removal of individuals.

The current study investigated the potential of common Pandora brood stock formation from 4 and 5 year old individuals (T.L. more than 300 mm) cultured in floating cages, which according to (LARRANETA, 1953; SELLAMI-ZRIBI, 1974; GHORBEL & KTARIM, 1982; MYTILINEOU, 1987) should have undergone sex reversal and be males. In addition, a comparison between natural spawning without hormonal treatment and spawning with the use of Human Chorionic Gonadotropin (HCG) was performed regarding the number of viable eggs produced.

Materials and Methods

The experiments were conducted both in the aquaculture laboratory of the Hellenic Centre for Marine Research (HCMR) and the 'Galaxidi Sea Farm' aquaculture farm in central Greece and form a succession of previous work done on this species (KLAOUDATOS *et al.*, 2004). The brood stock was supplied from cultured individuals reared in floating cages at the 'Galaxidi Sea Farm', aged between four and five years (more than 300 mm total length). There was an initial uncertainty for the presence of female individuals since, as mentioned in the literature, common Pandora occur in sizes below 220 mm (LARRANETA, 1953; SELLAMI-ZRIBI, 1974; GHORBEL & KTARIM, 1982; MYTILINEOU, 1987). In the first year of the research, four groups of brood stock were established, two of them at the 'Galaxidi Sea Farm' and the rest at the installations of the HCMR in the Saronikos Gulf. Following the first year of research, two more brood stock groups were established at the 'Galaxidi Sea Farm'. The groups formed at the HCMR underwent induced spawning with the use of Human Chorionic Gonadotropin (HCG) (250 and 500 IU/kg) (Table 1) (KLAOUDATOS *et al.*, 2004). Whereas all the groups formed at the 'Galaxidi Sea Farm' spawned without hormonal treatment (Table 2).

The main difficulty faced with the HCG hormone administration was the limited number of female individuals available. In

Table 1
Results of HCG hormone injection for groups 3, 4 (KLAOUDATOS *et al.*, 2004).

Treatment IU/Kg	Number of fish	Mean weight (g)	Fish responding	Responding Time (h)	Viable eggs (%)	Hatching (%)
500	15	815 ± 144	9	48	6.43	75
250	14	748.3 ± 130	9	52	15.0	76

addition, problems were encountered with the sedation of this species since the use of quinaldine resulted in sudden nervous behaviour by all individuals and the attempt to use benzokain (100-200ppm) or phenoxyethanol (200ppm) did not yield better results. The hormonal treatments applied are shown in Table 1.

The hormonal treatment was applied in order to attain the eggs during a small period of time since, under natural spawning without hormonal treatment; females produced small amounts of eggs over a lengthy period (Table 3). This lengthy egg production poses difficulties for a hatchery to collect and incubate an adequate number of eggs of the same age resulting in the coexistence of larvae of different sizes and cannibalism.

All groups of fish were initially bathed in a formaldehyde solution (200ppm) for one hour to reduce the possibility of parasitical infection. The tanks used for storing each group were cleaned, sterilized and covered after the fish transfer to keep the fish calm and eliminate the possibility of escape.

All groups at the 'Galaxidi Sea Farm' were kept in a rectangular plastic tank of 30m^3 volume at concentrations of 1.18 kg m^{-3}, and 1.25 kg m^{-3} for groups 1 and 2 respectively, whereas for groups 5 and 6 concentrations were 1.51 kg m^{-3} and 1.41 kg m^{-3}, respectively. Groups 3 and 4 established at the HCMR were transferred within two isothermic 2m^3 tanks at a concentration of 9.4 kg m^{-3}. The oxygen concentration was kept well above 8mg l^{-1} throughout the transfer and 2-phenoxyethanol was used as a sedative in concentration of 150ppm. The two groups formed at the HCMR were kept in two square cement tanks of 12m^3 volume at concentrations of 3.4 kg m-3 and 3.2 kg m^{-3}, respectively. All groups were acclimatized in their new environment

Table 2
Number of individuals (N), Body weight (B.W.), total length (T.L.), standard deviation (S.D.), population structure, and spawning induction for all groups formed.

Group No.	N	Location	B.W. (g) ± S.D.	T. L. (cm) ± S.D.	M / F	Spawning Induction
1	50	Galaxidi	705±115	33.9±2.2	2: 1	Natural
2	50	Galaxidi	735±102	31.5±1.5	2: 1	Natural
3	50	NCMR	815±144	35.1±1.8	3: 2	HCG
4	50	NCMR	760±160	34.7±2.1	4: 1	HCG
5	50	Galaxidi	910±155	37.1±1.8	2: 1	Natural
6	50	Galaxidi	850±92	35.8±2.3	2: 1	Natural

Table 3
Results of spawning under different spawning conditions for all groups formed during the course of the study.

Group No.	Number of Eggs Produced	% Eggs Viable	Viable Eggs(Day)$^{-1}$ (Female)$^{-1}$	Viable Eggs(Day)$^{-1}$ (Kg)$^{-1}$	Spawning induction
1	2,237,300	74.6	6,313	8,960	Natural
2	2,373,000	79.8	6,870	9,540	Natural
3	1,841,085	6.43	34,100	41,800	HCG
4	1,347,250	15.0	21,380	29,940	HCG
5	3,537,300	77.4	6,268	6,920	Natural
6	3,873,000	81.8	6,538	7,770	Natural

according to APOSTOLOPOULOS & KLAOUDATOS (1986).

Water supply at the 'Galaxidi Sea Farm' was kept at 1,200 l h^{-1}, and salinity at 37±0.7‰ for all groups, whereas at the HCMR the water supply was kept at 4,500 l h^{-1}, and salinity at 37±1‰. The supplied sea water was filtered (30-μm) and UV-sterilized, for all groups of fish. Oxygen was kept close to 100% saturation. The eggs for all groups were collected in a pot from the overflow of the tanks, using a net with mesh size of 600μm. The eggs were gently transferred to a 20 l clear volumetric cylinder and allowed to separate with the viable eggs floating and the dead and non-fertilised ones sinking according to (SMITH & HATAYA, 1982).

Results

All the brood stock groups formed resulted in viable egg release which was spontaneous (did not require stripping) while the proportion of females ranged between 20% and 40% in all groups. The total number of eggs produced, the percentage of viable eggs, the number of viable eggs produced per day per female and per kilogram of fish, and the spawning conditions are summarised in Table 3. In addition, the viable eggs produced per female and per kg of fish are shown in Figure 1. Egg collection from groups 1, 2, 5 and 6 that spawned under natural conditions started about 20 days after the acclimatization date. The egg production lasted for a mean period of 20 days after which it dropped significantly and stopped after the completion of one month from the onset of spawning for the groups that spawned under natural conditions without hormonal treatment. Whereas, egg production lasted for a total 6 and 7-day period for the third and fourth group, respectively, that spawned under hormonal treatment. During the spawning period, which ranged from the middle of May to the beginning of July there were several days for all groups during which egg production did not occur.

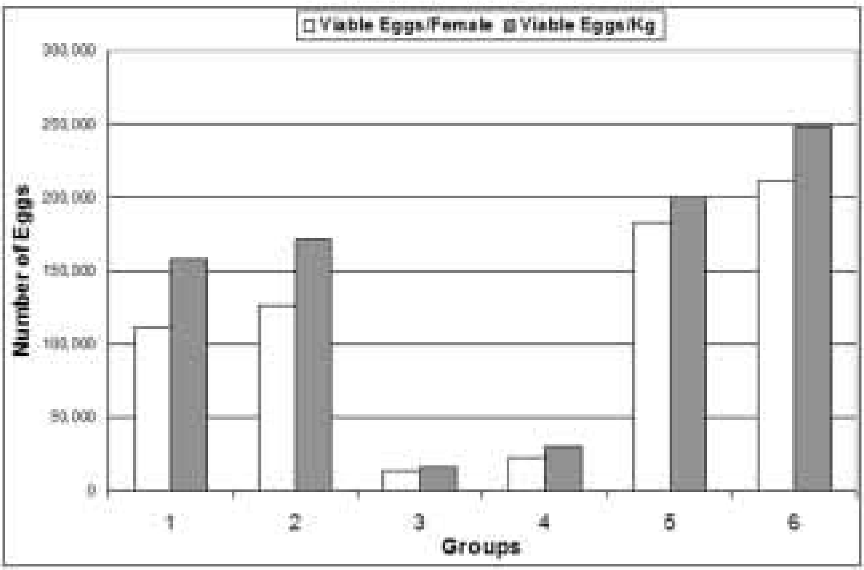

Fig. 1: Comparative bar chart of the number of viable eggs produced per female and per kilogram of fish for each of the six groups formed.

The hormonal treatment applied in groups 3 and 4 at the HCMR (Table 1) was used to induce spawning only in the female individuals, with gonads in the last vitellogenic state. In order to identify the appropriate individuals a biopsy was performed and showed non-synchronised ovarian development for this species indicating a prolonged spawning season (KLAOUDATOS *et al.*, 2004).

The hormonal treatment reduced the duration of the prolonged natural spawning period as the treated females released their eggs within a period of a few days. Statistical analysis was performed to check possible differences in the number of viable eggs produced between groups that spawned under natural environmental conditions and under hormonal treatment. The number of viable eggs produced in all four groups that spawned without hormonal treatment (groups 1, 2, 5, and 6) were checked for normal distribution. More than 50% of the data were normally distributed using the Anderson - Darling normality test (DYTHAM, 2003). The data also showed homogeneity of variance using Bartlett's test (Test Statistic 2.468, $P>0.05$). ANOVA (Analysis of Variance) was used to determine possible differences between the data. The results of ANOVA ($F=2.18$, $P>0.05$) indicated no significant difference in the number of viable eggs produced between all four groups that spawned without hormonal treatment.

The number of viable eggs produced between the two groups that spawned under hormonal treatment (groups 3 and 4) was normally distributed (Anderson - Darling normality test) and homogenous (Test Statistic 0.423, $P>0.05$). The results of ANOVA ($F=0.17$ $P>0.05$) indicated no significant difference in the number of viable eggs produced between the two groups that spawned under hormonal treatment.

Further analysis was performed in order to detect possible differences in the number of viable eggs produced between all groups. More than 50% of the data were approximately normally distributed (Anderson - Darling normality test) but significant heterogeneity of variance was indicated (Test Statistic 25.719, $P<0.001$). Data transformation was attempted and failed; as a result a non-parametric alternative test (Kruskal Wallis) was used for the comparison of all groups (DYTHAM, 2003). The results of the Kruskal Wallis Test indicated that the number of viable eggs produced were significantly different in at least one of the six groups ($H=37.13$, $P<0.001$). Dunn's method was used for all pair-wise comparisons between groups. A summary of the comparisons performed for all Groups is shown in (Table 4).

The statistical tests performed indicated the production of a significantly lower number of viable eggs in the groups that spawned under hormonal treatment compared to the groups

Table 4
Summary of the statistical tests performed to detect possible differences in the number of viable eggs produced between groups (ns:not significant, *:significant).

Group N	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1	-	ns	*	*	ns	ns
Group 2	ns	-	*	*	ns	ns
Group 3	*	*	-	ns	*	*
Group 4	*	*	ns	-	*	*
Group 5	ns	ns	*	*	-	ns
Group 6	ns	ns	*	*	ns	-

that spawned under natural environmental conditions as shown in Figure 2.

Discussion

The sex ratio in naturally occurring populations of the common Pandora was shown to be unbalanced and in favour of females in the population present off the Canary Islands, (PAJUELO & LORENZO, 1998), and in the Mediterranean (VASSILOPOULOU *et al.*, 1986). Maturity is reached between the second and third years of life (LARRANETA, 1967; GIRARDIN & QUIGNARD, 1985; MYTILINEOU, 1989; PAJUELO & LORENZO, 1998). Size at maturity, according to PAJUELO & LORENZO (1998), in naturally occurring populations off the Canary Islands is 174 mm and 232 mm total length for females and males, respectively. Several reports also mention that female common Pandora occur only in sizes below 220 mm total length (LARRANETA, 1953; SELLAMI-ZRIBI, 1974; GHORBEL & KTARIM, 1982; MYTILINEOU, 1987), while PAJUELO & LORENZO (1998) reported that sex reversal for the common Pandora in a naturally occurring population is complete, indicated by the absence of females in the older individuals.

The current study indicated that in captivity sex reversal is not complete shown by the fact that, despite the age of the brood stock being between 4 and 5 years old, and a total length in excess of 300 mm, a percentage ranging between 20 to 40% of the individuals were females. In many hermaphrodite species individuals change sex, not only because they have reached a particular size or age, but also due to behavioural or/and social (demographic) changes (SHAPIRO, 1992). Captivity could result in an intensification of behavioural signals by artificially creating such social groups. Sex change and sexual structure of populations could be also affected by environmental factors (CHAN & YEUNG, 1983; ZOHAR *et al.*, 1978; BUXTON & GARRATT, 1990).

Sex reversal of gilthead sea bream is conditioned by social and hormonal factors (MORETTI *et al.* 1999; ZOHAR *et al.*, 1978; BUXTON & GARRATT, 1990). Furthermore, during sex reversal a number of males under the presence of a large number of females do not undergo sex reversal but remain and

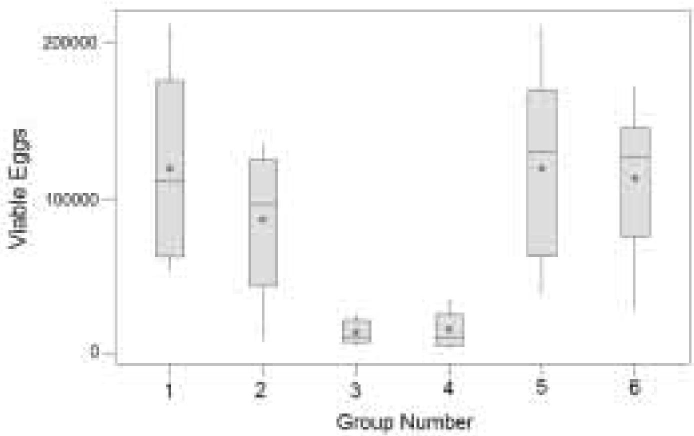

Fig. 2: Comparative box plot of the number of viable eggs produced from each group. Each box represents roughly the middle 50% of the data set. The lines extending to either side indicate the general extent of the data. The median value is marked inside the box, whereas the dots indicate the mean value.

develop as males (ZOHAR *et al.*, 1995). Sex reversal in Sparidae is an alternative reproductive style that enables individuals to maximise lifetime reproductive success by functioning initially in the early stages of their lives as a member of one sex and later on as a member of the opposite sex (ZOHAR *et al.*, 1978; BAXTON & GARRATT, 1990; KLAOUDATOS *et al.*, 1997).

According to HAPPE & ZOHAR (1988) sex reversal in the gilthead sea bream is largely controlled by the population structure and will only occur in the period between the end of the reproductive period and the beginning of the next, comprising the most critical period for the formation of a brood stock of a hermaphrodite species. Furthermore, a given brood stock of gilthead sea bream of the ratio of 1 male to 1 female will not remain constant over the years and the addition of young fish in older populations should be avoided, as it might induce older males to undergo sex reversal.

In groups formed during the current study the reproductive period started in the middle of May and ended at the beginning of July. In nature the reproductive period starts in April and ends in September (MYTILINEOU, 1989; VASSILOPOULOU & PAPACONSTANTINOU, 1990), with individuals in the state of maturity present even during the winter period (PAPACONSTANTINOU *et al.*, 1989).

The results of spawning in the groups that spawned without hormonal treatment indicated that the common Pandora is a sequential spawner as further indicated by uneven oocyte sizes in the ovary (KLAOUDATOS *et al.*, 2004). Sequential spawning occurring for an extensive period, in combination with the small number of continuous egg production, constitutes an advantage for a species ensuring high recruitment (SHAPIRO, 1992). Sequential spawning of a small number of eggs generates management problems for the hatchery regarding the proper planning of commercial fingerling production. Induced breeding represents, therefore, a choice for many commercial hatcheries in order to acquire the release of eggs in small periods of time. This was the main reason for the use of HCG in two of the groups formed. However, according to MORETTI *et al.* (1999) HCG treatment presents some serious drawbacks: not all females respond to it, egg quality may be below acceptable standards (hatching rate below 80%), being a large molecule provokes an immunization reaction, resulting in the fish treated with this hormone not responding when treated again the following season. Finally, it is less effective in inducing out-of-season spawning. HCG has, therefore, been successfully replaced by an analogue of the gonadotropin-releasing hormone (GnRH), a small molecule (10 amino acids) acting on the pituitary gland to induce the release of gonadotropins, which in turn act on the gonads (ZOHAR *et al.*, 1989; ZOHAR & MYLONAS, 2001). Furthermore, almost 100% of the injected fish spawn eggs whose quality usually matches that of natural spawnings and no immunization effect is reported.

In the current study the hormonal induced spawning resulted in the release of eggs during a small period of time, ideal for a hatchery that requires precise planning of year round egg and fry production. Nevertheless, the viability of the released eggs from the groups that spawned under hormonal treatment was significantly lower compared to the groups that spawned without hormonal treatment. In addition, the eggs produced from all groups per kg per day were low compared to the egg production from domesticated gilthead sea bream of 2-3 million eggs per kg per season (ZOHAR *et al.*, 1995). However, early efforts to induce spawning in the gilthead sea bream was rarely spontaneous from the captive brood stock resulting in a low number of eggs released. This was probably a result of domestication, a process that naturally occurs in captive brood stocks over generations (ZOHAR *et al.*, 1995). Similarly, it is expected that through domestication of common

Pandora the number of eggs produced will substantially increase.

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