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SHORT COMMUNICATION

Induced spawning in common sole (*Solea solea* L.)

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In Europe, the soles *Solea solea* (Linnaeus 1758) and *Solea senegalensis* (Kaup 1858) are considered among the most promising species for marine fish farming. Several studies conducted from the 1970s to develop the production technology of these species suggested simple schedules to obtain spontaneous spawning (see references in Baynes, Howell & Beard 1993). Despite these results and available technical information, nowadays, sole production is still at the pilot scale as breeders in captivity perform poorly or fail to spawn (Imsland, Foss, Conceição, Dinis, Delbare, Scram, Kamstra, Rema & White 2004).

Moreover, for mass production essential knowledge of some reproductive traits, such as ovulatory period, spawn frequency, egg production and male fertility, is still scarce.

With the aim of producing juveniles to supplement natural recruitment, a broodstock of *S. solea* was adapted to captivity and induced to reproduce. The present study reports the results of 5 years of experiments.

Five hundred and sixteen soles were caught by trawling in the Gulf of Venice (Northern Adriatic Sea, Italy) in 2 consecutive years (1998–1999) and maintained in a local fish farm (Pellestrina, Veneto Agricoltura). All breeders were tagged with passive integrating transponders and after 1 year survivals of the two groups were 29% and 28% respectively. Soles were kept under a natural photoperiod in a 30 m^2 (27 m^3) tank with a sand bottom (10 cm layer) and recirculating seawater at 35 $\pm$ 0.6 g L^{-1} salinity. Water temperature fluctuated according to the natural seasonal trend, but was maintained above 8 °C from November to March, and below 25 °C during summer. The mean monthly temperatures averaged over 1999–2003 years (January–December) are shown in Fig. 1. The tank stocking density was 0.1–0.6 kg m^{-2}, corresponding to 1–3 individuals m^{-2}.

Breeders were fed *ad libitum* (1–5% body weight) three to five times a week, with an experimental moist feed composed of fish meal, raw molluscs (mussels and scallops), shrimp, cod-liver oil and vitamins. During spawning, the diet was replaced by scallops and polychaetes (Ramos 1986).

Sole breeders were measured every year before and after the spawning season, and in winter during gonadal development. Condition factor (K) and relative growth increment (RGI) were also calculated. Gonadal ripeness was checked at individual level through the transponder.

In the first year of rearing, the broodstock reacted positively to captivity and, because of the applied feeding and rearing schedules, an RGI of 59 $\pm$ 28% for females and 30 $\pm$ 25% for males was observed from spring to autumn.

As an effect of gonadal maturation in both sexes, RGI calculated from winter to spring generally showed an average positive trend, with higher values

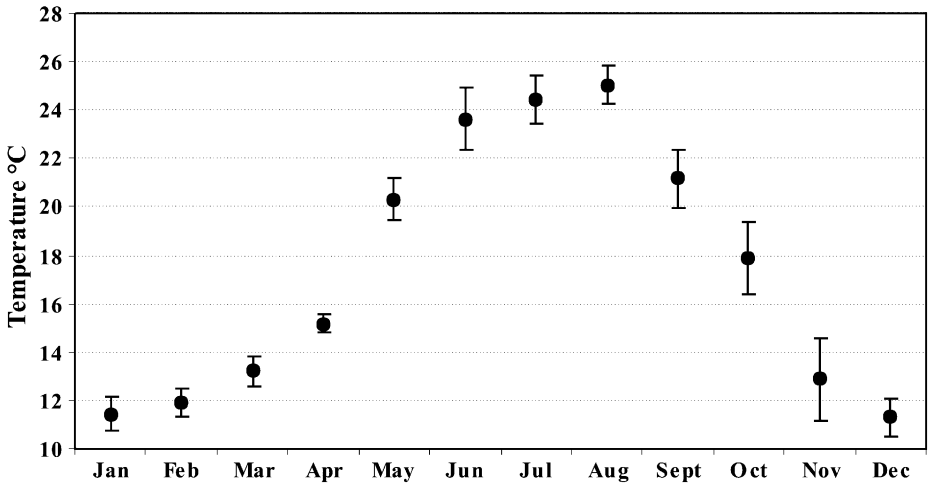

Figure 1 Average monthly water temperature during the years 1999–2003. Data are expressed as means ± 95% confidence limits.

in females (13.9–33.9%) than in males (9.7–25.6%) in each of the 5 years except 2002. In that year, RGI values were exceptionally low (− 1.8% for males and 5.2% for females) probably as a consequence of feeding competition, as already reported for the sole by Howell (1997).

Soles captured from the wild spontaneously spawned within the first year of captivity, from March to May. Some of the breeders captured in 1998 spawned in 2 consecutive years.

In spring 2000, as soon as the first eggs were released in the stocking tank, two lots of fish were transferred to spawning tanks. Breeders were selected to form homogenous mating lots on the basis of gonad shape as visible externally or in transparency against a strong light. Each lot was placed in a 9 m^2 (10 m^3) spawning tank with recirculating water, sandy bottom and natural photoperiod. The tank density was 0.1–0.8 kg m^{-2}, and the sex ratio was close to one (Table 1).

With the aim of improving egg production, a commercial preparation of a long-acting agonist of gonadotropin releasing hormone (GnRHa) (Enantone Depot, Takeda, Japan) was used to induce spawning (Barbaro, Francescon, Bertotto, Bozzato, Di Maria, Patarnello, Furlan & Colombo 2002). Long-acting GnRHa was chosen as it induced successful production of eggs both in single batch group synchronous and in asynchronous species (Mylonas & Zohar 2001). The hormone was diluted in physiological saline and administered both in females and males by a single intramuscular injection.

In 2000, one lot (40A) was injected with a dose of 40 μg kg^{-1} BW, while the other one was allowed to spawn spontaneously and kept as control (Table 1 and Fig. 2). In both lots, spawning started within 84 h and continued for about 2 months. Eggs were collected daily within 12 h from spawning, transferred to 100 L incubators and counted as described by Chatain and Gauvrit (1994).

In the following year (2001), as no spontaneous egg release was detected throughout March, in April two lots (lot 40B and lot 20A) were injected with 40 and 20 μg kg^{-1} BW of GnRHa respectively. Data on egg production in 2000 and 2001 are reported in Table 1.

With regard to egg viability in 2000, the treated lot (40A) and the control showed a significant difference in production of developing embryos with 2217 vs. 5111 per kg BW per day respectively (ANOVA: $F = 18.7$; $^*P < 0.05$). Lot 40A produced as many eggs as the untreated control but about 80% of the eggs were laid in the first 22 days (Fig. 2a, b).

Lot 40B (2001) produced fewer eggs than 40A but with a higher fertilization rate, indicating a better quality of gametes. Deterioration of gamete quality was related to hormone dosage for *S. solea* (Ramos 1986) as well as for other species, and was linked to drastic shortage of ovulation period after induction (Mylonas, Hinshaw & Sullivan 1992): a similar situation seemed to characterize lot 40A in which hormone dosage was probably too high and injection was administered at an unsuitable stage of ovarian maturation.

In 2001, the lot treated with 20 μg kg^{-1} (20A) produced twice the embryo amount of 40B lot (ANOVA: $F = 5.2$; $^*P < 0.05$), thus indicating that such a dose was indeed able to induce spawning (Table 1).

Table 1 Biometry and egg production in sole experimental lots

	Control: 2000	Lot 40A: 2000	Lot 40B: 2001	Lot 20A: 2001	Lot 20B: 2002	Lot 20C: 2002	Lot 20D: 2003	S 20A: 2003	S 20B: 2003
Breeders									
Females									
N of individuals	10	11	7	7	9	9	5	1	1
Total length (cm)	27 ± 2	28 ± 2	32 ± 1	32 ± 2	35 ± 2	35 ± 1	35 ± 6	39	37
Weight (g)	228 ± 72	228 ± 64	413 ± 71	425 ± 52	556 ± 91	563 ± 56	581 ± 282	808	750
K (condition factor)	1.0 ± 0.2	1.0 ± 0.1	1.3 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4	1.5
Biomass (g)	2276	2506	2890	2978	5006	5066	2906	808	750
Males									
N of individuals	9	9	8	8	7	7	6	1	1
Total length (cm)	27 ± 2	27 ± 2	30 ± 3	30 ± 2	32 ± 3	32 ± 3	31 ± 7	36	35
Weight (g)	189 ± 46	194 ± 53	272 ± 91	279 ± 55	362 ± 135	335 ± 105	380 ± 268	436	428
K (condition factor)	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	1.0	1.0
Density (kg mq^{-1})	0.5	0.5	0.6	0.6	0.8	0.7	0.6	0.1	0.1
Sex ratio (F/M)	1.1	1.2	0.9	0.9	1.3	1.3	0.8	1.0	1.0
Oviposition									
Total laid eggs (1000 kg^{-1})	321	347	163	302	394	>85	507	373	270
Developing embryos (1000kg^{-1} and (%))	256 (79)	89 (34)	118 (67)	238 (74)	220 (59)	6 (7)	213 (45)	232 (54)	0 (0)
Egg diameter (μm)	961 ± 7	968 ± 9	1004 ± 10	989 ± 11	1015 ± 19	1061 ± 22	1108 ± 9	962 ± 7	1008 ± 9
Spawning period (days)	69	55	24	25	71	>22	43	58	73
Oviposition days	50	40	20	22	45	>18	34	22	27

Data are expressed as means ± 95% confidence limits.
K (condition factor): body weight*100/total length^3.
Sex ratio, N of females/N of males.

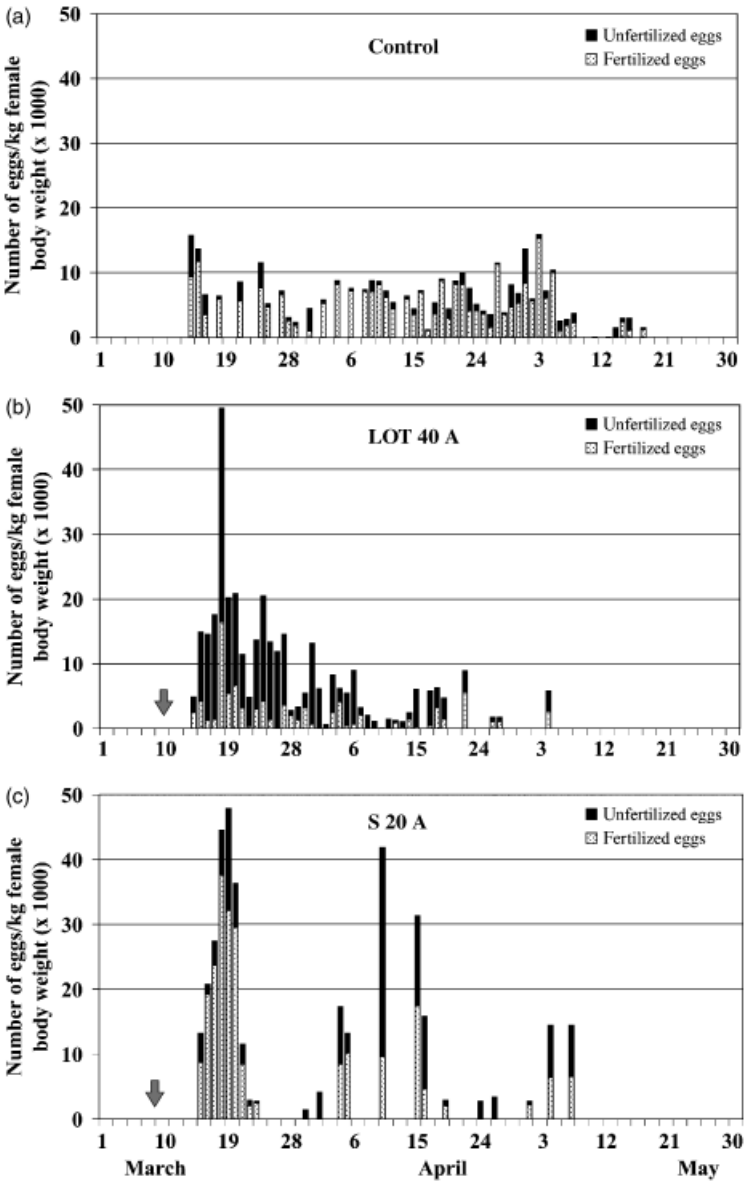

Figure 2 Daily total egg production in sole lots: (a) a control lot (only environmental induction), (b) a lot treated with 40 μg kg^{-1} BW of long-acting GnRHa, (c) a single female treated with 20 μg kg^{-1} BW of long-acting GnRHa. Arrows indicate the time of hormone injection.

In March 2002, after spontaneous spawning failed again, two lots were injected with 20 μg kg^{-1} BW GnRHa (lot 20B and lot 20C; Table 1). Lot 20C produced almost exclusively unfertilized eggs and after 1 month, to attempt further exploitation of the breeders, three single mating pairs were set-up without any further hormonal injection. The single mating pairs spawned intermittently 3, 12 and 22 days during about 2 months of observation. The daily egg production ranged from 3800 to 70 000 per kg BW.

Spawning occurred for periods ranging from 2 to 8 consecutive days, and the latency interval between each spawning was 1–19 days. Only one single mate pair produced fertilized eggs.

In the following year (2003) when again no spontaneous eggs were found, a multiple lot (20D) and two single mating pairs (S20A and S20B) were injected with hormone preparation. Egg production was successfully achieved in all lots, but in one single pair fertilization completely failed (Table 1). Single females released eggs consecutively for periods ran-

ging from 2 to 9 days with latency intervals of 1–11 days (Fig. 2c representing only S20A), giving an indication about the rhythm of ovulation in *S. solea* under hormonal treatment.

Sole breeders studied herein spontaneously spawned within the first year of captivity and eventually spawned in the following year. However, in contrast to that reported by Devauchelle, Alexandre, Le Corre and Letty (1987) and Lenzi and Salvatori (1989), prolonged captivity apparently inhibited egg release.

When egg release was inhibited in the stock tank, it always started in the experimental groups just after administration of long-acting GnRHa yielding productions comparable with spontaneous spawning, although concentrated in a shorter period.

The effectiveness of long-acting GnRHa is further highlighted by comparison with data on egg production reported in the literature for soles spontaneously spawning (range 3000–240 000 embryos per kg BW per season) (Houghton, Last & Bromley 1985; Ramos 1986; Devauchelle *et al*. 1987; Baynes *et al*. 1993) as well as in short-acting LHRHa-induced females (31 900 embryos per kg BW per season) (Ramos 1986).

Apart from hormonal treatment, other parameters such as diet and water temperature are of paramount importance in promoting proper gonadal development and spawning in captive soles. Administration of low food rations to sole breeders during the winter of 2001–2002 gave rise to strong competition that led to extremely low RGIs, with special reference to males, and a consequent decrease in fertilization rates in laid eggs. Moreover, variability in fertilization rates in the present investigation may be explained by male infertility, a frequent problem in flatfish reproduction (Mylonas & Zohar 2001).

Reproduction in common sole still requires further study, particularly regarding some peculiar aspects such as improvement in male performances in fertilization, identification of effective minimal hormonal doses and set-up of methods for gamete manipulation. The present work may be considered as a further contribution to this topic.

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