Animal Biology, Vol. 55, No. 3, pp. 203-218 (2005)

Also available online - www.brill.nl

Gonad development and spawning behaviour of artificially-matured European eel (*Anguilla anguilla* L.)

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Abstract—Gonadal development and spawning behaviour of artificially-matured European eel (*Anguilla anguilla* L.) was studied. Treatment of males with Human Chorionic Gonadotropin (HCG; 1 IU g/week) resulted in a Gonado-Somatic Index (GSI) of 10.88 ± 3.39 and spermiation. Treatment of females with carp Pituitary suspension (cPs) (20 mg cPs/kg body weight per week) resulted in oogenesis with a GSI of 20.0 ± 11.3 ($n = 7$), and the number of eggs per female was $1874 * 10^3 \pm 1116 * 10^3$; ($n = 7$). Ovulation of the females was induced with $17\alpha, 20\beta$ dihydroxyprogesteron (DHP) at 2 μg/g bodyweight. Eggs of European eel were found to be non-sticky and typically pelagic. Maximum speed of eggs rising to the surface in a water column was 2.24 ± 0.33 metres (m) per hour (h). To study behaviour in a qualitative way, two females were used together with three groups of three males. During a 283 minute (min) observation of the two females, we observed female-female interaction: 'lethargic behaviour' (33.6%) vs. 'cruising together' (66.4%). In the period when males and females were together (188 min), we observed 'approaching the head region of the female' (57.7%), 'touching the operculum' (39.4%), or 'approaching the urogenital area' (2.9%) by the males (total 725 seconds (s)). Sperm release in the presence of a female took 115 s of the total approaching time of 725 s (15.9%), while in the case of male-male interaction this was only 15 s of the total period of 116 s (12.9%). Induced spawning behaviour of eels was collective and simultaneous, corresponding to spawning in a group. This is the first time group spawning behaviour has ever been observed and recorded in eels.

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INTRODUCTION

When eels migrate to the ocean in the autumn, their gonads are regressed. If they are kept in aquaria, there is no further gonadal development. Based on these observations, it seems that maturation can be triggered by environmental factors during migration. Until now, the nature of these factors was unknown. Some of these possible external factors are: i) hydrostatic pressure (Nilsson et al., 1981; Fontaine et al., 1984; Dufour and Fontaine, 1985; Sebert and Barthelemy, 1985; Simon et al., 1988); ii) low temperature (Nilsson et al., 1981); or iii) swim exercise (Palstra et al., unpubl.; van Ginneken et al., unpubl.). It has recently been demonstrated, in European eels in the silver stage, that there is a prepubertal blockage of gonadal maturation at the neuroendocrine level because of a deficiency of gonadotropin-releasing hormone (GnRH) at the level of the pituitary, resulting in a lack of production of gonadotropin (GTH). Furthermore, there is an inhibition of GTH release by dopamine (Dufour, 1994). Both factors are responsible for an immature gonad.

The main obstacle to successful aquaculture of European eels is the production of viable eggs and larvae. For reproduction of most fish species, only the final part of the gametogenesis, final oocyte maturation and ovulation, has to be induced. In contrast, the entire reproduction cycle, including endogenous and exogenous vitellogenesis and also ovulation, has to be induced in eels.

Several authors applied the protocol for artificial administration of hormones. If we consider the research on this topic chronologically, much attention has been paid to the production of gametes and the production of viable larvae, while little study has been carried out on behavioural aspects of mating eels.

As early as 1936, Fontaine obtained sperm from male eels treated with Human Chorionic Gonadotropin (HCG; Fontaine, 1936). Several other authors also performed artificial stimulation of sperm production (Boetius and Boetius, 1967; Dollerup and Graver, 1985; Miura et al., 1991). Yamamoto and Yamauchi were the first to obtain eel larvae from *Anguilla japonica* (Yamamoto and Yamauchi, 1974; Yamauchi et al., 1976). Boetius and Boetius (1980) injected *A. anguilla* with pituitary extract and published a photograph of a spermiating male close to a swimming mature female. Bezdenezhnykh and Prokhorchik described embryonic and post-embryonic development of European eel larvae until 3.5 days post-hatch. However, details about the methods were not published in their papers (Bezdenezhnykh et al., 1983; Prokhorchik 1986; Prokhorchik et al., 1987). Ohta et al. (1996) showed that the addition of Dihydroxyprogesterone (DHP) is crucial for the induction of ovulation. Lokman and Young (2000) applied the procedures of Ohta (1996) to the New Zealand eel (*A. dieffenbachi*, 5 kg) and demonstrated that DHP also induced ovulation in this species. Pedersen et al. (2003) applied the procedures of Ohta (1996) to Japanese eel (*A. japonica*) and, using the same procedures, also succeeded in producing larvae from European eel (*A. anguilla)* (Pedersen, 2003, 2004). Recently, our group was able to fertilise egg batches of nine females and the development of

1600 embryos was followed until 4 days after fertilisation (Palstra et al., in press). Hatching was not observed.

From the above studies, it is clear that induced sexual maturation of European, Japanese, and New Zealand eels may lead to fertilised eggs and viable larvae. However, when the procedure of 'stripping' (removing sexual products from the animals by gentle pressure to the abdomen) is applied, natural behaviour does not occur and spawning behaviour cannot be studied. Therefore, we hypothesise that if artificially matured eels are put together in an aquarium, spawning behaviour can be observed. Until now, the spawning behaviour of eels has never been described, because the spawning grounds of American and European eel species are in the Sargasso Sea, several thousand kilometres (km) away from land. Our laboratory approach will give new insights into the life cycle and the mating behaviour of this catadromic fish species.

MATERIAL AND METHODS

Hormones and chemicals

To induce oogenesis in females, carp pituitaries were obtained from a commercial company 'Catfish', (Den Bosch, The Netherlands). Ovulation was induced in females with 4-pregnene-17α, 20β-diol-3-one (17α, 20β dihydroxyprogesteron (DHP)) (Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands). To induce spermiation in males, Human Chorionic Gonadotropin (HCG) was used (Sigma Aldrich Chemie BV).

Preparation of the hormone solutions

For the preparation of the carp pituitary extract, portions of 0.5 g carp pituitaries were weighed and each portion was ground with a pestle in 5 ml saline solution (9% NaCl). The suspensions were supplemented with 10 ml buffered saline and homogenised with an ultratorax (2 $\times$ 5 s at 3000 rpm). Next, the 'carp Pituitary suspension' (cPs), was treated for 10 min in an ultrasound bath (2-4°C). After 12 h storage on ice, the suspension was centrifuged for 10 min at 20000 g, 4°C. Next, the supernatant was pipetted, recentrifuged and stored at -80°C in 1 ml aliquots. The final ratio of carp pituitary vs. buffered saline solution was 1 g of carp pituitary suspended in 40 ml saline solution.

For the preparation of the ovulation hormone, 10 mg of DHP was dissolved in 875 μl 100% ethanol. Of this solution, 175 μl per kg was diluted with saline solution (1:1) and injected intraperitoneally at several locations in the ovary.

Growth of the ovary with cPs and the induction of ovulation

Nineteen females (range 0.7-2.5 kg), each identified with microchips (Trovan), were kept in a recirculation system of 1000 l artificial seawater (33 promille) at 18°C.

The eels were caught in the autumn of 2000 in lake Grevelingen, The Netherlands, during their seaward migration. The maturation experiments were performed in the period April-August, 2001. Ovarian growth of each animal was stimulated by weekly IP injections of 20 mg cPs/kg body weight. On the day of the injection, and to prevent bacterial infections, the animals were exposed to the antibiotic Flumequine (50 mg/L) for 3 h in a separate tank. Body weight was measured. After 8 weeks (eight injections), body weight was measured 2 days after the injection. Two days after cPs injection, body weight increased by more than 10%. At this point, a sample of the eggs was taken with a cannula and investigated under a binocular microscope. If the eggs were opaque, the female was not ready to ovulate. When most eggs had undergone final oocyte maturation, signified by their becoming transparent, the female was selected for the ovulation protocol. After 14 weeks of treatment, the first females were ready for the induction of final oocyte maturation. Most females had mature oocytes after 14-25 injections of HCG. When a female was selected after injection with dihydroxyprogesterone (DHP) together with a 'booster' injection of cPs (20 mg cPs/kg body weight), she was placed in a 1500 l aquarium at 20°C (temperature shock of 2°C). Ovulation could be observed after 16-24 h by gently pressing on the belly of the female. In fertilisation experiments, ovulated eggs were stripped without force. Of the 19 females used in the experiment, seven females were finally selected for ovulation, while two females were used to film spawning behaviour. Ten females died during the injection protocol. In order to avoid handling stress during the whole injection protocol over the period of 14 weeks, animals were anaesthetised on the day of injection in a solution of 3-aminobenzoate ethyl ester methanesulphonate (MS-222, Sigma, St Louis, MO, USA) buffered with $NaHCO_3$ at a final concentration of 200 ppm.

Sperm production

In the autumn of 2000 approximately 100 male eels (100-150 g body weight) were caught by a local fisherman at lake Grevelingen during the eels' seaward migration. Weekly injection of the males started in April 2001. In order to exclude pheromone effects, they were kept apart from the females in a re-circulating system of 1500 l of artificial seawater (33 promille). Concerning water quality, the maximum values for NH_4, NO_2, and NO_3 were 0, 0 and 50 mg, respectively, per litre, at 18°C.

Growth of the testis of each animal was induced by weekly IP injections with Human Chorionic Gonadotropin (HCG) at a dosage of 1 IU/g body weight per week (Ohta et al., 1997). The first males released sperm after 6 weeks. Sperm motility in seawater was checked under a microscope. Also, the body weights of males were recorded each week.

Speed of eggs rising to the surface

In six cases, released water-activated eggs rising in the water column were quickly transferred to a 2000 ml glass beaker. Each individual egg was released at the 500 ml

bar and the required time for the egg to rise up to the 1000 ml bar (= 6.4 cm) was measured. From these data speeds were calculated and averages±standard deviation were expressed in m/h and m/day.

Experimental set-up for spawning behaviour

Two chronological protocols were used to observe and study spawning behaviour. To achieve this, the eels were allowed to swim freely in a 4000 l aquarium. In the control protocol, two females were used. To study spawning behaviour, the same two females, followed by three sequential batches each of three males, were used. Our observation period of only female-female interaction lasted for 283 min. The period of female-male and male-male interaction lasted 188 min. The first batch of three males was for a period of 83 min with the two females, the second batch of three males for a period of 50 min, and the third set of three males for a period of 55 min. We used indirect light so as not to disturb the eels. We could follow the experiment on monitors outside the laboratory. A Sony camera was used to record the spawning behaviour of the eels on film.

Calculations and statistics

Statistics were performed using a one-way ANOVA. $P \leqslant 0.05$ was considered as statistically significant. Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov and $F_{\max}$ tests, respectively.

RESULTS

At the start of the experiment in April 2001, a control group of males was sampled and several zootechnical parameters were measured. The first HCG-treated males gave sperm as early as 6 weeks. After 12 weeks, 75 males gave 1-1.5 ml sperm per animal per week. The other 25 males did not respond to the hormone treatment and gave no sperm. In August 2001, after the maturation experiments were finished, 18 males that had been treated with hormones were still alive: mortality was high due to long-term hormonal treatment. The GSI of these animals was measured (see table 1). Figure 1 shows three mature males with developed gonads.

At the start of the experiment in April 2001, a control group of females was sampled and several zootechnical parameters were measured before treating the females with cPs. No placebo-treated animals were studied because the injection with saline has no effect on gonad development for males (Boetius and Boetius, 1967) or females (Boetius and Boetius, 1980). Of the initial 19 females, six died during the first 14 injections while four others died at a later stage due to unknown causes. Of the 11 silver eels, which received at least 16 injections, seven matured and were treated with DHP. When the increase in bodyweight exceeded 10% of the original weight (Ohta et al., 1996), the animal was selected to induce ovulation via DHP injection.

Table 1.
Zoological parameters of Control- and HCG-treated males. Of the latter group eleven animals reacted positively on the hormone treated with HCG and produced sperm while seven HCG-treated males reacted negatively on the hormone treatment.

	A: Control ($n = 12$)	B: HCG-treated Positive responders ($n = 11$)	C: HCG-treated Negative responders ($n = 7$)	P-value A-B	P-value B-C	P-value A-C
Bodyweight (g)	132.94 (45.02)	82.55 (18.42)	72.50 (11.76)	0.002*	0.249	0.004**
Length (cm)	39.38 (1.79)	38.23 (2.33)	38.50 (2.59)	0.059	0.827	0.187
Gonad weight (g)	0.27 (0.26)	9.41 (4.68)	0.47 (0.27)	0.0001**	0.0001**	0.150
GSI	0.18 (0.116)	10.88 (3.79)	0.63 (0.34)	0.0001**	0.0001**	0.0001**

P-values are given comparing groups: A-B, B-C and A-C.
* Denotes significantly different ($P \leqslant 0.05$) from Control group.
** Denotes significantly different ($P \leqslant 0.001$) from Control group.

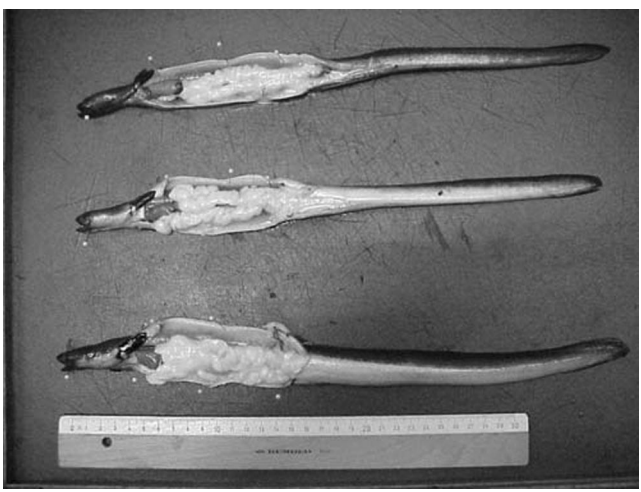

Figure 1. HCG-treated mature males with a mean gonadosomatix index (GSI) of 10.9 ± 3.79.

Seven females were stripped and zootechnical parameters of seven stripped females are presented in table 2. Apart from GSI, the number of eggs was also determined. Figure 2 shows a mature female with a GSI of 40.2. Nuclear migration in an eel oocyte is illustrated in figure 3 (Germinal Vesicle Migration; GVM). Other characters of a ripe female were the swollen soft belly and a strong penetrating odour.

Eggs rose to the surface at a speed of 1.58 ± 0.78 mh^{-1} (one female: six trials with stripped and fertilised eggs); however, individual differences were still high. The three fastest eggs rose at a speed of 2.24 ± 0.33 mh^{-1} or 53.7 ± 8.0 mday^{-1}. Eggs were free rising to the surface and did not stick to the sides or to each other.

Three types of spawning behaviour were observed (fig. 4).

Table 2.
Gonad maturation parameters of a Control-female group of the European eel (*Anguilla anguilla* L.) and of a cPS-treated group.

	Bodyweight (g)	Length (cm)	Gonad weight (g)	GSI	Weight egg (mg)	Number of eggs × 1000
Control-females Mean ± (SD)	1091.8 (132.2)	78.4 (4.37)	17.02 (4.76)	1.54 (0.30)	XXX	XXX
cPs-treated females						
1	884	70	245	27.71	0.121	2,026
2	1664	85.5	399	23.98	0.101	3,945
3	656	71	63	9.60	0.067	950
4	744	71.5	73	9.81	0.068	1,066
5	746	69	96	12.87	0.124	772
6	1701	87	683	40.15	0.269	2,532
7	872	73.5	139	15.94	0.076	1,830
Mean ± (SD)	1038.14 (447.28)	75.36 (7.581)	242.57 (227.91)	20.01 (11.26)	0.118 (0.071)	1,874 (1,115)
P-value Control vs. cPs	$P \leqslant 0.907$	$P \leqslant 0.650$	$P \leqslant 0.001^{**}$	$P \leqslant 0.001^{**}$		

* Denotes significantly different ($P \leqslant 0.05$) from Control group.
** Denotes significantly different ($P \leqslant 0.001$) from Control group.

Female-Female Interactions

During a period of 283 min, female-female interactions were observed (fig. 4D). For the first 5 min, both females actively swam in the aquarium. Thereafter, a period followed during which both females hung lethargically at the water surface. During periods of activity (totalling 95 min), one female approached the other towards the head region. Both females stayed together and swam together head-to-head for a certain period of time near the surface of the water (fig. 4D). The relative percentage of 'lethargic behaviour' vs. 'cruising together' was 33.6% and 66.4%, respectively.

Male-Female Interactions

The individual observations of behaviour, split up for the three consecutive groups of three males (repetitions), are given in table 3 and figure 5. An hourly average was taken of the number of male visits made to different regions (operculum, head and urogenital) of the females. This was 8.7, 18.0 and 7.6 visits/h for male groups A, B and C, respectively. The total time spent visiting the different body regions of the female (operculum, head, urogenital region) was 144.6, 360 and 245.45 s/h for male groups 1, 2 and 3, respectively (table 3).

We observed sperm release by several males with one female (fig. 4C). After an initial orientation phase of several minutes, males swam vigorously around pursuing a female, swimming parallel to her, and pushing her (fig. 4A). Usually, the male

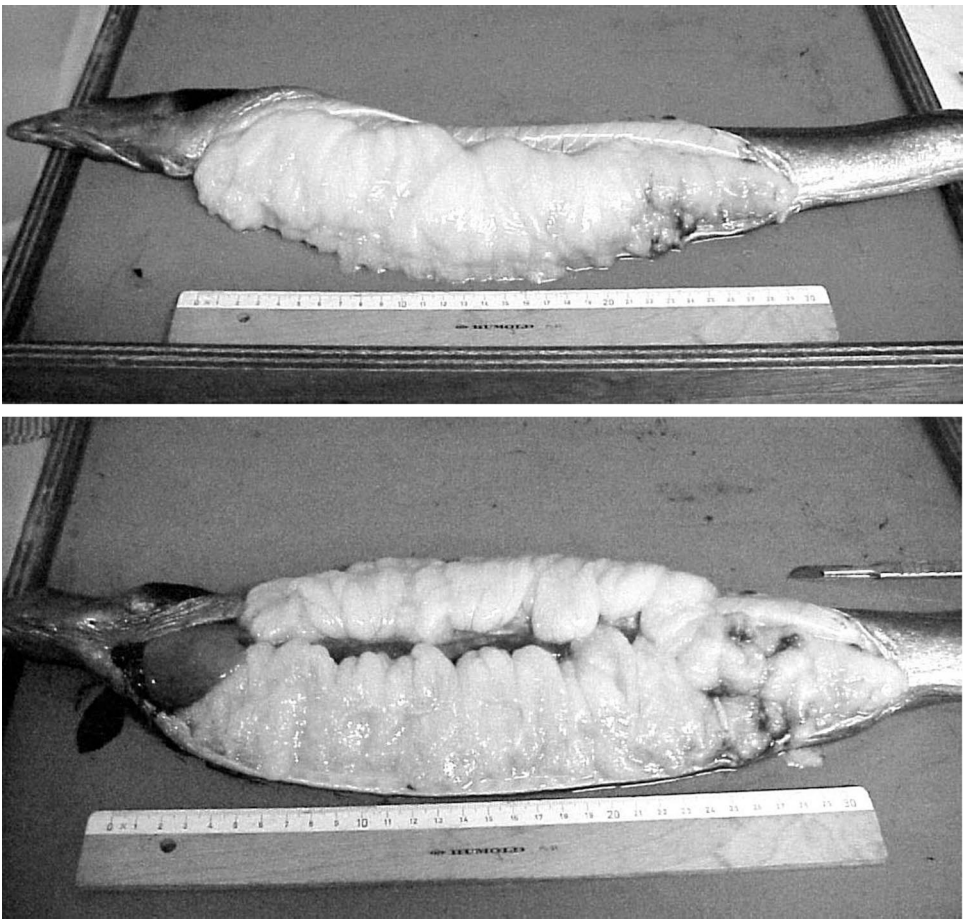

Figure 2. Mature female of European eel with GSI of 40.2.

approached the head region of the female and chased her for a short period of time, constantly touching and butting the operculum of the female with his head (fig. 4A). After this, the male took on an S-like body shape, while ejaculating sperm near the urogenital region of the female (fig. 4C), and swam away. The males repeated the chasing and the sperm ejaculation several times and released sperm at all regions of the female (head, operculum, urogenital region). On a few occasions, the male pushed at the urogenital region of the female with his head followed by the sperm release. Sometimes males ejaculated sperm, assuming an S-like shape. In these cases, they swam parallel to a female, with or without briefly butting her near the urogenital region.

However, in most cases, the male did not assume this S-like body shape, but instead ejaculated his sperm as he passed by. On some occasions, a male chased a female while touching her operculum, after which the male swam away again, not releasing any sperm. In percentages, the different forms of behaviour can

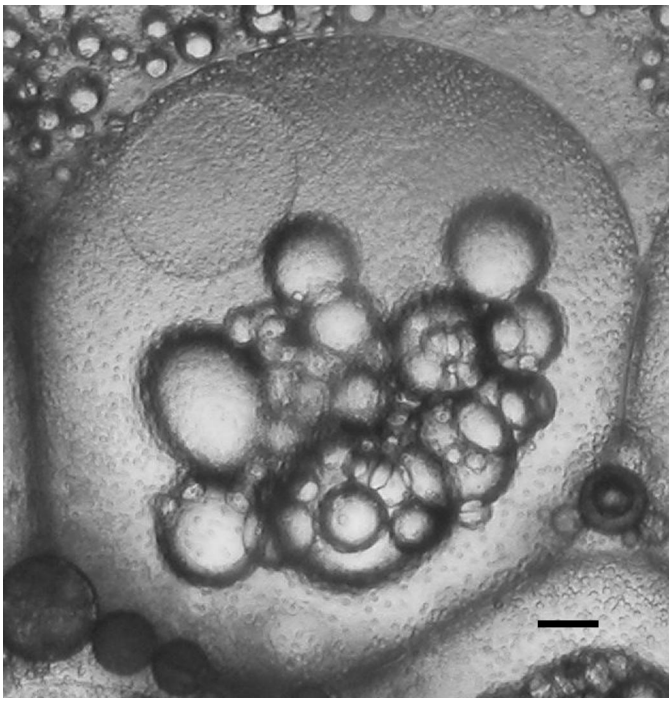

Figure 3. Oocyte showing Germinal Vescicle Migration (GVM) (scale bar = 100 μm) (phase contrast microscopy).

be classified as follows: i) approaching the head region of the female (57.7%); ii) touching the operculum (39.4%); iii) or approaching the urogenital area (2.9%) by the males (fig. 5). Occasionally, interaction of two males with one female was observed (fig. 4A). Both males chased the female by touching her operculum while ejaculating sperm. The females also became active due to the spawning activities of the males.

During the total observation period of 188 min, males approached the female several times, covering a period of 725 s. During this period, males ejaculated sperm during 115 s, which corresponded to 15.9% of the male-female approaching time. Sperm ejaculation occurred with all three sets of males. In the second set of three males, eggs were visible at the urogenital region of both females. With the last set of three males, one female released eggs. The males released so much sperm that no clear film images could be made due to turbidity. To overcome this problem, we had to put a protein skimmer on our filter system in order to clear the water. Males were also attracted to the eggs on the floor of the aquarium, and to the urogenital tract of the female when the female released eggs. When the floor was totally covered with eggs, they did no longer contact the females who stayed at the water surface.

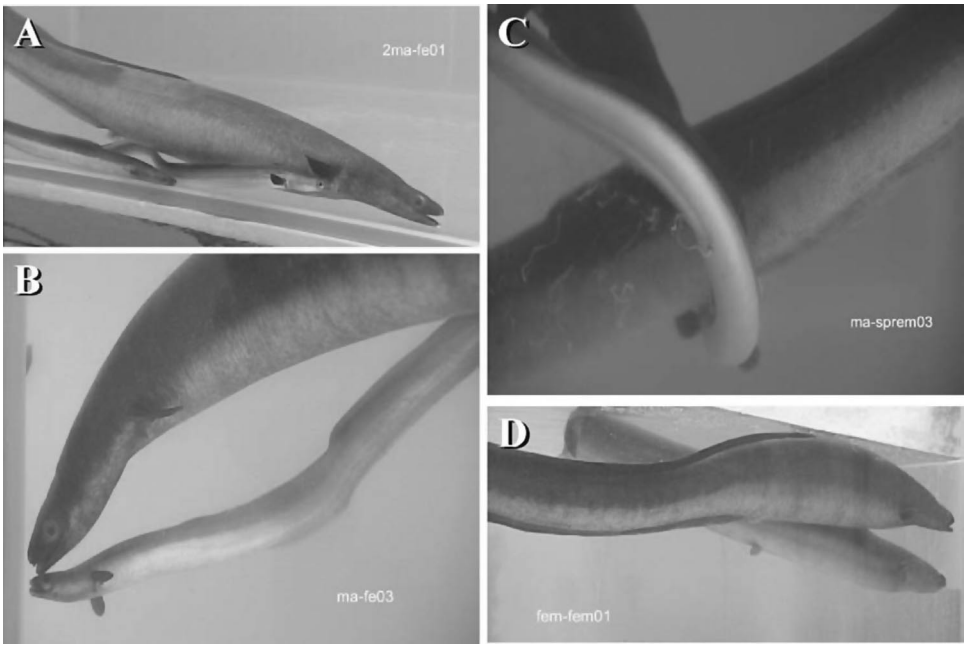

Figure 4. Spawning behaviour of artificially-matured European eel (*Anguilla anguilla* L.) was studied. Two females were used, together with successively three groups of three males to record their spawning behaviour in the 4000 l aquarium; **A:** Male touching female at gill area; **B:** Male stimulates female at the head region; **C:** The release of sperm by two males with one female; **D:** Female-female interaction, females chase each other.

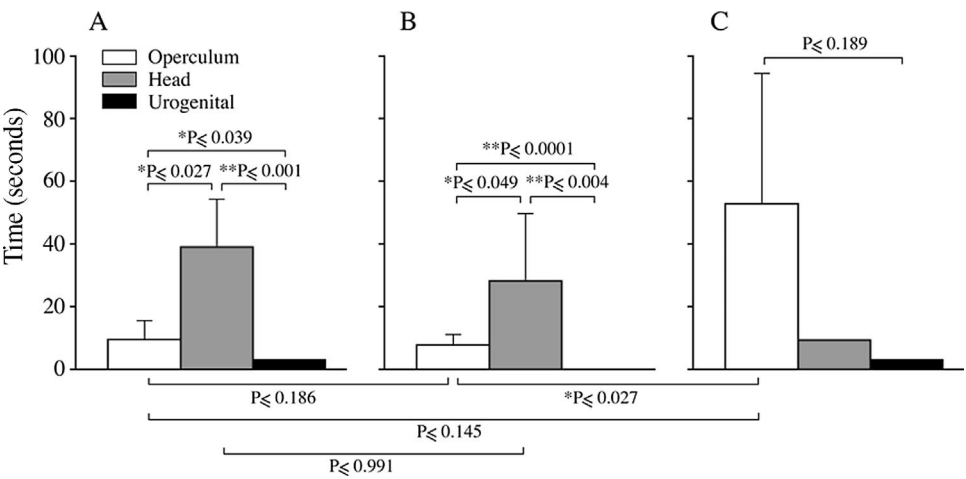

Figure 5. Female-seeking behaviour of males. Mature males exposed to two ovulating females approached females at three different regions: the operculum, the head region or the urogenital area. The mean duration in seconds of each type of behaviour is shown in three consecutive trials with three males each (A, B, C).

Table 3.
Female-seeking behaviour of males. Mature males exposed to ovulating females approached these at three different regions: the operculum, the head region or the urogenital area. Data from three consecutive trials (A, B, C), each with three males and two ovulating females.

	A: 1st group of males (observation period: 83 min)			B: 2nd group of males (observation period: 50 min)			C: 3rd group of males (observation period: 55 min)		
	Operculum	Head	Urogenital	Operculum	Head	Urogenital	Operculum	Head	Urogenital
Individual	4	31	3	6	13	0	30	9	3
visit	9	40	3	6	11	0	32		3
duration*	16	60	3	10	114	0	33		
		25	3	13	28	0	115		
			3	4	37	0			
				8	18	0			
					28	0			
					24	0			
					80	0			
Mean±SD*	9.67 ± 6.03	39.0 ± 15.30	3.0 ± 0	7.83 ± 3.25	28.11 ± 21.24	0	52.5 ∓ 41.68	9	3.0 ± 0
Visit frequency**		8.67			18.0			7.64	
Duration of visits***		144.58			360.0			245.45	

* Individual visit duration and mean duration of each type of visit, in seconds.
** For each trial, the visit frequency (mean number of visits per hour) to the three regions.
*** For each trial, the total duration of visits (in seconds per hour) to the three regions.

Male-male interactions

Males chased each other, only releasing sperm in a few cases. Sperm release lasted only 15 s (12.9%) of the total period of 116 s of male-male interaction. During ejaculation, males showed the same body movement (S-shape) as during sperm release in a male-female interaction.

DISCUSSION

Spawning behaviour and gonadal development of artificially-matured European eel (*Anguilla anguila* L.) was studied in a 4000 l aquarium. During the period of female-female interaction we distinguished: 'lethargic behaviour' versus 'cruising together'. In the period of combined male and female interactions we observed 'approaching the head region of the female', 'touching the operculum', or 'approaching the urogenital area' by the males. Males reacted immediately after transfer.

In this study on *Anguilla anguilla*, female GSI values ranged between 9.6 and 40.2; this maximal value is lower than the 60.7 reported by Boetius and Boetius (1980) for the same species. The highest GSI values recorded for female Japanese eels, *A. japonica*, treated with salmon pituitary extract, were around 70 (Yamamoto and Yamauchi, 1974; Sugimoto et al., 1976). The highest GSI reported for female American eels, *A. rostrata*, treated with cPs, was 44.8 (Edel, 1975). No GSIs were given in the literature for *A. australis* and *A. dieffenbachi* (Lokman and Young, 2000). It is important to indicate whether the GSI was determined before or after ovulation. If it is determined after Germinal Vesicle Migration (GVM) and Germinal Vesicle Breakdown (GVBD), the volume of the ovary will be much higher due to the uptake of water by the oocytes.

Based on oocyte weight and GSI, we calculated that egg numbers were in the range of 772 000-3945 000 eggs per female. Dekker (2000) estimated, based on fisheries assessment data for the European eel stock, a recruitment of 2000 million glass eels. Based on our fecundity data, an estimation of the total number of spawners needed in the Sargasso Sea to maintain the standing stock at the European continent is difficult to give because data on larval mortality during hatching in the Sargasso Sea followed by oceanic migration are lacking. It is more than likely that only a small fraction of the total number of eggs produced become reproductive adults that migrate back to the Sargasso Sea.

In our study, the first males released sperm after 6 weeks of treatment with HCG. The rate of maturation is probably dose-dependent, which is confirmed by data in the literature. Male *A. anguilla*, given a dose of HCG twice as high as in our protocol, became mature after 8 weeks (Eckstein et al., 1982). Probably, the maturation rate is also species-dependent because *A. japonica* males, given the same dosage as in our experiment, became mature after 9-12 weeks (Ohta et al., 1996). It is also possible that within a certain eel species temporal aspects and the maturation status of the animal might also be important and vary throughout the year. For the

European eel the animals with the most progessive stage of development of gonads are found in fall (unpubl. results) and that is why we collected our animals in this period prior to their seaward migration. So, maturation rate may not only be species-dependent, but also reflect slightly different times during the annual cycle in which the hormones were administered.

The small and numerous eggs of European eel were found to be non-sticky and typically pelagic. Therefore it is assumed that eggs will not stick to seaweed such as *Sargassum*, typical for the Sargasso Sea. Pronounced hydration of eggs was observed in this study; this is typically seen in marine teleosts spawning pelagic (or buoyant) eggs (Wallace and Selman, 1981). The acquisition of buoyancy in pelagic spawners is a key event in reproduction and affects both fertility and survival of spawned eggs (Carnevali et al., 1999). In this study, eggs were found to rise in the water column up to the surface with maximum speeds of 2.24 m/h. Main hatching times for European eel are between 47 and 60 h after fertilisation (Bezdenezhnykh et al., 1983; Pedersen, 2003, 2004; Palstra et al., 2004a, b). During these times eggs will rise 105-134 m. Assuming that hatching occurs in the food-rich upper water layers these shallow depths may represent spawning depths. However, for Japanese eel it was observed that high pressure delays embryonic development and hatching times (Hiroi et al., 2003).

Little is known about the Sargasso Sea, an enormous potential spawning area of 2×10^6 km^2. At this moment, no information is available from the natural situation with regard to effects of population density, predation, light conditions, number of spawners, etc.

In a review, van Ginneken (unpubl. results), summarised what is known about an estimation of the number of eel spawners, observations made on hormone-treated females in the Sargasso Sea that were followed by telemetry tracking, and the catch of adult spawners on their way to the Sargasso Sea. We are aware that the present study, in a 4000 l aquarium, with two females and three groups of three males, has strong limitations in replicating the natural situation. However, this study is valuable because it is the first time that spawning behaviour of eels has ever been observed and described.

In the Sargasso Sea, sexual activity is probably limited to a restricted period. Therefore, the location of suitable mates, courtship and spawning must take place quite rapidly. It is likely that spawning is thus synchronised throughout the population. Information on the spawning behaviour of European eel has to our knowledge never been reported before. The ‘strong penetrating odour’, excreted by a ripe female may contain a pheromone that induces spawning behaviour. Natural spawning may be stimulated due to the action of pheromones (Colombo et al., 1982) which may induce ovulation and have a positive effect on hatching and fertilisation rates due to a synchrony of gamete emission after massive and collective spawning of eels.

Females were lethargic and waiting in the upper part of the aquarium to spawn. After an initial orientation phase, males periodically swam close to the females to

court them. Also, we observed, for European eel, that spawning behaviour started immediately after transfer and was synchronous, showing several forms of social interactions: male-male, male-female and female-female. For the male-female interaction, spawning of one female with several males occurred occasionally. Several forms of behaviour were observed including: i) lethargic behaviour: low activity as the consequence of the absence of a partner; ii) chasing behaviour: which includes chasing of another fish without reciprocation; iii) submissive behaviour: which we defined as the fleeing from an aggressive opponent; and iv) cruising: swimming over an area without being chased or followed.

In the initial phase of the experiment, before males were introduced in the aquarium, females were hanging lethargically at the surface of the aquarium. After the introduction of males, the females became more active. The males mainly demonstrated aggressive behaviour. For male-male interactions there were no observations of males defending a specific territory, or specific dominance among males, or of males maintaining space around them. In a few cases during male-male interaction, when males chased each other, this behaviour was followed by the release of sperm as was observed in male-female interaction. During male-female interaction, females demonstrated mainly submissive behaviour. Non-adhesive, pelagic eggs were released to the surrounding water and the parents showed absence of parental care. Therefore their behaviour can be classified as non-guarding. During our experiment, spawning took place in full light, and males were attracted to the eggs (without eating them) probably by odour. Therefore, crepuscular spawning which takes place with other fish species to prevent predation on zygotes (Roberts and Ormond, 1992) is probably not important for the European eel.

During female-female interaction we observed mainly cruising behaviour. Both females stimulated each other by swimming close to each other and touching each other. This behaviour may be important to induce a synchrony of gamete emmision and induce a massive simultaneous spawning of numerous mating eels in the Sargasso Sea.

ACKNOWLEDGMENTS

We thank Drs Lex Raat and Jan Klein Breteler, the Organization for Improvement of Inland Fisheries, Nieuwegein, Netherlands, for supporting this project by a grant of LNV. Dr Ir. Marc Lokman, Zoology Department, University of Otago, Dunedin, New Zealand, is kindly acknowledged for his help and advice, Dr Ingo Schlupp, University of Hamburg, Germany, for helpful advice and fruitful discussions on ethological issues and Dr Herman Berkhoud and Jos Onderwater for the beautiful pictures. Furthermore, the authors wish to thank Edwin Cohen and Eugenia Clavero for assistance. We thank Professor Dr M. Richardson for critically reading the manuscript and for helpful suggestions for improving the English. The eel migration project at the University of Leiden was supported by a grant

from the Technology Foundation (STW), which is subsidised by the Netherlands Organization for Scientific Research (NWO), STW-project no. LBI66.4199.

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