Aquaculture Research

Aquaculture Research, 2015, 1–9 doi: 10.1111/are.12755

Controlled reproduction in *Anguilla anguilla* (L.): comparison between spontaneous spawning and stripping-insemination approaches

Andrea Di Biase[1], Antonio Casalini[1], Pietro Emmanuele[1], Michaela Mandelli[1], Pieter Mark Lokman[2] & Oliviero Mordenti[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, Cesenatico (FC), Italy

[2]Department of Zoology, University of Otago, Dunedin, New Zealand

Correspondence: A Di Biase, Department of Veterinary Medical Sciences, Bologna University, Viale Vespucci 2, Cesenatico (FC) 47042, Italy. E-mail: andrea.dibiase2@unibo.it

Abstract

This study aimed to compare the fertility of eggs between artificially matured female silver eels that spawned spontaneously and those that were spawned by manual stripping. The effects of the two methods of spawning on ovulation and fertilization rate were also investigated. For this purpose, 18 wild female European eels captured in *Bonello* lagoon (North Adriatic Sea) were carp pituitary extract-injected to undergo sexual maturation and ovulation; a final injection of 17,20β-dihydroxy-4-pregnen-3-one (DHP) was administered when at least 30% of the oocytes were fully transparent. After the DHP-injection, nine eels were transferred to a new closed recirculating aquaculture system, where they were housed with spermiating males (sex ratio 4/1) to allow spontaneous spawning (SPT-group); the remaining nine eels were transferred to a 250 L tank and ovulation was checked at four-hourly intervals by manual stripping (STR-group). The number of eggs per female in the SPT-group was significantly greater than that in the STR-group. Furthermore, fertilization rates in the SPT-group were notably higher than those observed in the STR-group. Significantly, the best performances were obtained among eels in which at least 50% of oocytes were fully transparent at the time DHP was administered. We conclude that the fertility of eggs from spontaneously spawning eels is superior to that of eggs acquired by strip-spawning and artificial fertilization.

Keywords: European eel (*Anguilla anguilla*), artificial reproduction, egg production, egg quality

Introduction

The eel is among the highest-value species produced from freshwater aquaculture in the world. To date, all seedlings for cultivation originate from wild glass eels or elvers collected in estuarine waters. However, over the last several decades, natural stocks of eels, especially those representing the commercially valuable temperate species such as European eel (*Anguilla anguilla*), American eel (*Anguilla rostrata*) and Japanese eel (*Anguilla japonica*), have decreased markedly (Casselman 2003; Dekker 2003; Tatsukawa 2003) due to overfishing, environmental destruction, oceanographic/climatic changes and other as yet unknown factors (van Ginneken & Maes 2005; EELREP 2006). Indeed, *A. anguilla* is now included in the IUCN Red List of critically endangered species (Freyhof & Kottelat 2010). Unfortunately, this measure has not reduced the risk of extinction (Mordenti, Di Biase, Sirri, Modugno & Tasselli 2012). One effective way to preserve these species is through the establishment of techniques for mass production of glass eels to reduce the fishing pressure on wild glass eel stocks (Okamura, Horie, Mikawa, Yamada & Tsukamoto 2013).

With reference to the European eel, research has focused on successful artificial propagation protocols in terms of hormone dose and timing and with regard to defining optimal environmental parameters (water temperature, water salinity and

photoperiod; Durif, Dufour & Elie 2006; Mordenti, Di Biase, Bastone, Sirri, Zaccaroni & Parmeggiani 2013) to obtain a high number of eggs for artificial fertilization. Nevertheless, one of the major problems of seed production remains the constant availability of high quality eggs. Egg quality, in terms of resulting fertilization, hatching and survival rates, is highly variable between batches from different individuals (Chai, Tosaka, Abe, Sago, Sago, Hatanaka, Ijiri & Adachi 2010) or between different egg retrieval methods (Mordenti, Casalini, Mandelli & Di Biase 2014). Thus, it is necessary to improve techniques for the production of high quality eggs to make mass production of eel larvae possible. The use of 15–25 weekly injections of salmon or carp pituitary extract (CPE) can advance oocytes to the migratory nucleus stage after which final oocyte maturation and ovulation are typically induced by 17,20β-dihydroxy-4-pregnen-3-one (DHP)(Kagawa, Tanaka, Ohta, Okuzawa & Hirose 1995; Ohta, Kagawa, Tanaka, Okuzawa & Hirose 1996; Ohta, Kagawa, Tanaka, Okuzawa & Iinuma1997; Palstra, Cohen, Niemantsverdriet, van Ginneken & van den Thillart 2005; Mordenti *et al.* 2012, 2013). However, for this routine method, the relationship between timing of the DHP-induced final stage and the resulting egg quality remains to be explored. The objective of this study was to investigate the quality and quantity of eggs produced by artificially matured European eels (*A. anguilla*) that were allowed to spawn spontaneously or that were subjected to stripping-insemination, and to relate these fertility parameters to the timing of DHP administration.

Materials and methods

Animal source and maintenance

Wild female eels were caught using traditional *lavoriero* (downstream trap) in a brackish water lagoon near the sluices of the North Adriatic Sea (*Val Bonello*, Veneto - Italy). The *Val Bonello* (50 ha) is a closed lagoon located in the Po Delta, where a fishery has been operating for centuries, taking advantage of the autumn–winter migration of euryhaline fish to the sea: several species thrive in the *Valli*, but the fishery has always been dominated by *A. anguilla*. Large female eels were selected from the catch and then transported to the laboratory. Cultivated male eels (n = 50 fish, 118–237 g in BW), reared in freshwater, were purchased from a commercial supplier at the same time, and upon transfer to the laboratory, gradually acclimated to seawater over 7 days. All eels were kept in a recirculating system consisting of two fish-rearing tanks (700 L), one with females and one with males; fish were maintained in complete darkness (-0.04×10^3 lux at the bottom of the tank without water; Mordenti *et al.* 2012) in seawater (salinity 32 g L^{-1}) at a controlled temperature of 15.5 ± 0.5°C until gonadal maturation (see sections 'induction of maturation in female eels' and 'induction of maturation in male eels', below) was complete. This system was equipped with a foam separation tank (protein skimmer) and a biological filter containing plastic porous balls. The tank was also provided with a thermal regulation system, a UV-sterilizer lamp, an ozonizer and an aerator (electromagnetic air compressor) to adjust the rearing water conditions.

Induction of maturation in female eels

At the experimental premises, the female eels were measured and sampled to obtain an external indicator of their maturation state (silver index) (Durif, Dufour & Elie 2005; Di Biase, Bastone, Casalini, Parmeggiani, Costantini & Mordenti 2012) and their condition factor (K) was calculated according to the formula below:

$$K = (BW * BL^{-3}) * 10^6$$

BW: body weight (g), BL: body length (mm).

Nine females were selected for artificial maturation (see below) and subsequent spontaneous spawning (*SPT-group*) and another nine for egg retrieval by strip-spawning (*STR-group*); only eels with comparable body weights (600 ± 100 g) were used for artificial reproduction. The animals were marked individually by inserting fish tags (FLOY TAG Mod Floy T-Bar Anchor) in the dorsal muscle while under anaesthesia with 400 ppm 2-phenoxyethanol, and maintained under starvation for the duration of the trial. Once a week, females received an intramuscular injection with CPEs at a dosage of 10 mg kg^{-1} BW (1st–3rd week), 20 mg kg^{-1} BW (4th–6th week), 30 mg kg^{-1} BW (7th–9th week) or 40 mg kg^{-1} BW (10th week-final maturation) (Mordenti *et al.* 2012). Weekly administrations of CPE continued until the

beginning of oocyte hydration, i.e. until the BW exceeded 110% of initial body weight (IBW), similar to what has been done by many other researchers (Dou, Yamada, Okamura, Shinoda, Tanaka & Tsukamoto 2008; Oliveira & Hable 2010; Burgerhout, Brittijn, Kurwie, Decker, Dirks, Palstra, Spaink & van den Thillart 2011; Ijiri, Tsukamoto, Chow, Kurogi, Adachi & Tanaka 2011; Mordenti *et al.* 2013). However, the timing of the subsequent DHP injection was notably changed – thus, rather than administering DHP at a fixed time after the last CPE injection, the timing was optimized for individual eels. To this end, females were repeatedly ovary-biopsied (~0.3 mL, equating to ca. 500 follicles) every 8 h by needle and syringe while under anaesthesia in 400 ppm 2-phenoxyethanol. Thereafter, once at least 30% of the oocytes were fully transparent, displaying their nucleus at the periphery and containing few large fat droplets (diameter from 110 to 150 μm; i.e. fully transparent oocytes, FTO), ovulation was induced by intra-peritoneal DHP injection (2 mg kg^{-1}). The developmental stage of the FTO corresponded to stage 5 of gamete development in *A. anguilla* according to Palstra *et al.* (2005) and to stage 7 in *A. japonica* according to Unuma, Hasegawa, Sawaguchi, Tanaka, Matsubara, Nomura and Tanaka (2011). In case, the desired stage of oocyte maturation (stage 5 according to Palstra *et al.* 2005) was not reached within 48 h from the last routine weekly CPE injection, a booster with CPE (i.m. 40 mg kg^{-1} BW) was administered and ovarian biopsies were again taken as described above to properly time the injection with DHP. The body weight at the final CPE administration, whether a routine weekly or a follow-up booster injection, was used to calculate the body weight index (BWI), as follows:

$$\text{Body weight Index (BWI)} = (\text{BW IBW}^{-1}) * 100$$

BW: body weight at DHP (g), IBW: initial body weight (g).

Induction of maturation in male eels

Males were induced to mature following standard protocols (Ohta *et al.* 1997; Palstra *et al.* 2005); briefly, they were injected with 1 IU g^{-1} BW hCG and started spermiation after a 5-week treatment. Just before fertilization experiments, the males received a booster hCG injection (1 IU g^{-1} BW) to induce sperm maturation (Burgerhout *et al.* 2011). Sperm motility was monitored and only males with at least 50% sperm motility (continuous activity of >50% of spermatozoa) were used for experimentation (Burgerhout *et al.* 2011).

Experimental design: effects of insemination method on reproductive parameters

Eighteen females were matured and either allowed to spawn spontaneously (*SPT-group*; $n = 9$) or used for egg retrieval by strip-spawning (*STR-group*; $n = 9$); after DHP injection, each female of the *SPT-group* was transferred to a new closed recirculating aquaculture system, in which the seawater temperature was raised to 20 ± 0.5°C (Dou *et al.* 2008; Mordenti *et al.* 2014) and maintained for 20 h in the company of spermiating males (*sex ratio* 4M/1F) to facilitate spontaneous spawning. The system, composed of one spawning chamber, two incubation chambers and one outlet chamber, has been described in more detail in Mordenti *et al.* (2014). After 20 h, all breeders were removed from the spawning chamber. In contrast, DHP injection of each eel in the *STR-group* was followed by transfer to a 250 L tank supplied with recirculating seawater at a temperature of 20 ± 0.5°C. The artificial fertilization programme started 8 h post DHP injection when females were assessed for ovulation at 4-hourly intervals (8, 12, 16 and 20 h) by applying gentle pressure on the abdomen in a cranial-to-caudal direction (Ohta *et al.* 1996); eggs were collected into a 3-L plastic sterilized bowl. Four males per female were hand stripped and milt was collected in a syringe (10 mL) and kept in the refrigerator for a maximum of 12 h. The collected sperm was mixed with 100 mL fresh seawater and then added to the dry eggs in the bowl. After approximately 3–4 min, the eggs were placed into buckets with fresh sterile seawater (~20 L) for 15 min. Each inseminated batch was kept in a 150 L polyethylene tank and maintained at the same temperature used to induce ovulation (20 ± 0.5°C).

Analyses: reproductive performance

For each spawning event, the relative weight of spawned eggs (%BW) was calculated as the difference in body weight post spawning and that at the time of DHP injection. The total fertilization rate (%) for each batch of spawned eggs was

observed at 2 h post fertilization and determined by calculating the % of eggs that reached the 8-cell stage; for this purpose, three subsamples of 1000 eggs were scored and averaged for each batch. Furthermore the fertilized, floating rate (%) was assessed on buoyant eggs, obtained after maintaining an egg sample for 30 min in a 500 mL beaker; again, only embryos in the 8-cell or 16-cell states were considered as fertilized. A check on fertilization success was also made on the sunken eggs.

All fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 86/609/EEC). Approval for this study was obtained by the Ethics Committee of Bologna University.

Statistic analysis

Reproductive performances were statistically analysed: statistics were performed using a *t*-test following a Student's *t*-distribution; $P \leq 0.01$ was considered statistically significant.

Results

The internal and external indicators of maturation state of the wild eels at the beginning of the experiment (T0) are reported in Table 1. All eels were silver, actively migrant and had a maximum silvering score (V) (only one eel was at stage IV).

Reproductive performance

SPT-group females ovulated between the 15th and the 28th week while in the STR-group, ovulation started at the 17th week and finished at the 29th week. One female/group (STR-8 and SPT-5) did not spawn, but retained eggs in the abdominal cavity (Table 2). There were no differences (d.f. = 16; $t_{Student} = 0.54$; $P = 0.60$) between both experimental groups with regard to the % of oocytes at stage 5 (FTO) prior to DHP administration, mean values hovering around 50%. Similarly, the BWI of SPT- (121.35 ± 7.44%) and STR-groups (119.88 ± 7.36%), obtained after DHP injection showed no evident differences from a statistical point of view (d.f. = 16; $t_{Student} = 0.42$; $P = 0.68$).

The females that spawned spontaneously (SPT-group) were statistically more productive (d.f. = 14; $t_{Student} = 0.92$; $P < 0.01$) than those stripped manually (Table 2). In both groups, a positive correlation (STR-group: $R^2 = 0.7595$; STP-group: $R^2 = 0.7749$; Fig. 1) was observed between the FTO and the relative weight of spawned eggs: in fact, the two females that retained the eggs (STR-8 and SPT-5) displayed the lowest percentage of FTO at the time of DHP injection within their respective groups (33.5% in STR-8 and 32.4% in SPT-5). Likewise, the most productive females had the highest percentage of FTO (Table 2).

In both experimental groups, fertilized eggs were obtained: in the STR-group, fertilization was successful for all females (eight eels; i.e. excluding STR-8 and SPT-5), while it was successful for six of 8 SPT-group females. The eggs from the SPT-three and SPT-7 females, although released spontaneously, were not fertilized as the males did not emit milt while being co-housed with the females in the spawning chamber (Table 3); moreover, courtship behaviour was not observed.

The total fertilization rate of the eggs of SPT females was higher (38.25%) than that from eels that were spawned by stripping-insemination (7.70%) (d.f. = 14; $t_{Student} = 3.46$; $P < 0.01$). Similarly, the rate of fertilized, floating eggs (Table 3) was significantly different between the two experimental groups (d.f. = 14; $t_{Student} = 3.06$; $P < 0.01$), edging higher in the SPT-group. Unlike floating eggs, sunken eggs were not fertilized for any of the females under study. When excluding the two spawning events with null outcome (SPT-3 and SPT-7), the total fertilization rate and that for the floating eggs in the SPT-group were 51% and 78%, respectively, notably higher than the corresponding values of 8% and 18% in the STR-group (Table 3).

Table 1 Characteristics of silver female European eels adopted for artificial maturation experiments

	STR-group (n = 9)	SPT-group (n = 9)
Eels		
Body weight (g)	598.42 ± 54.99	566.71 ± 63.35
Body length (cm)	69.60 ± 2.17	67.69 ± 2.98
Condition factor (K)	1.78 ± 0.12	1.83 ± 0.17
Silver index	V	IV-V

Discussion

Fertilization, hatching and survival rates can be highly variable between eggs from different

Table 2 Reproductive performance of artificially matured female European eels that were strip-spawned (STR-group) or that spawned spontaneously (SPT-group) after hormone treatment (see text for details)

	BWI (% BW)	Oocytes (% FTO)	Ovulation (Yes/No)	Spawned eggs (% BW)
STR-group				
1	122.2	61.4	Yes	32.40
2	108.3	40.4	Yes	24.50
3	115.5	37.5	Yes	12.70
4	128.5	68.4	Yes	38.40
5	112.8	63.2	Yes	30.80
6	118.1	58.3	Yes	33.80
7	131.3	38.5	Yes	25.40
8	123.7	33.5	No	–
9	118.5	41.5	Yes	23.60
Mean	119.88 ± 7.36	49.90 ± 13.37		27.70 ± 7.93
SPT-group				
1	117.7	64.5	Yes	42.50
2	116.7	62.3	Yes	47.50
3	118.5	35.8	Yes	39.60
4	124.9	70.5	Yes	52.80
5	134.1	32.4	No	–
6	107.9	59.2	Yes	44.50
7	125.8	38.9	Yes	40.40
8	126.2	40.2	Yes	37.60
9	120.4	72.3	Yes	50.20
Mean	121.35 ± 7.44	52.90 ± 15.88		44.39 ± 5.38*

*Significant difference ($P < 0.01$) between eels in the STR-group and the SPT-group. BWI: body weight index (increase in relative body weight after injection of fish with 17,20β-dihydroxy-4-pregnen-3-one); FTO: fully transparent oocytes at the time of treatment with 17,20β-dihydroxy-4-pregnen-3-one.

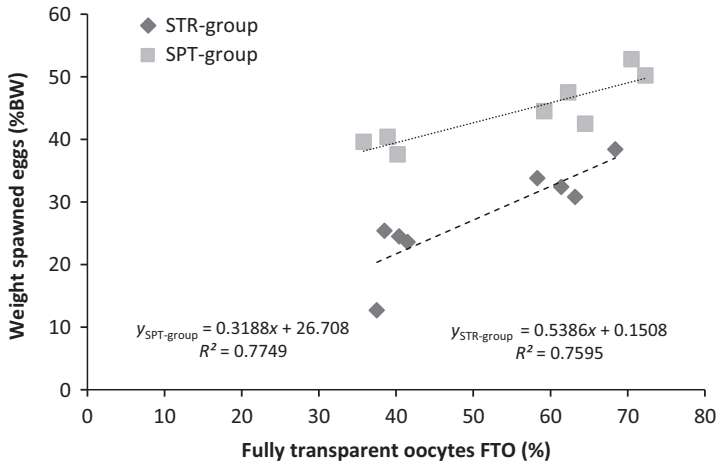

Figure 1 Relationship between the percentage of fully transparent oocytes (% FTO) and the weight of spawned eggs (%BW) from female European eels that spawned spontaneously (SPT) or that were spawned by stripping (STR).

individuals (Chai *et al.* 2010) or between different methods of egg retrieval (Mordenti *et al.* 2014). Although pituitary extract-induced resumption of oogenesis in captive silver eels can advance oocyte development to the migratory nucleus stage, ovulation and final maturation are routinely induced by DHP. However, the relationship between the timing of the induction of the final stages of development by DHP and the resulting quality of the eggs needs to be further explored. Accordingly, we administered DHP to artificially matured European eels (*A. anguilla*) whose ovarian follicles had varying % of fully hydrated oocytes and evaluated the resulting fertility after spontaneous spawning or in response to stripping-insemination.

Table 3 Fertilization rates of eggs from artificially matured female European eels that were strip-spawned (STR-Group) or that spawned spontaneously (SPT-group) after hormone treatment (see text for details)

	Fertilization		
	(Yes/No)	Total (%)	Floating (%)
STR-Group			
1	Yes	8.20	21.50
2	Yes	3.90	10.80
3	Yes	2.90	18.70
4	Yes	9.70	13.50
5	Yes	11.10	27.80
6	Yes	8.60	16.70
7	Yes	7.40	20.20
8	–	–	–
9	Yes	9.80	15.40
Mean		7.70 ± 2.89	18.07 ± 5.27
SPT-Group			
1	Yes	64.20	93.20
2	Yes	52.30	74.50
3	No	0.00	0.00
4	Yes	57.30	78.60
5	–	–	–
6	Yes	44.70	81.20
7	No	0.00	0.00
8	Yes	38.90	68.40
9	Yes	48.60	70.70
Mean		38.25 ± 24.81*	58.32 ± 36.78*

*Significance difference ($P < 0.01$) between eels in the STR-group and the SPT-group.

Silver eels captured in the wild have been mostly used as broodstock for induced maturation trials because under conditions of captivity in aquaculture ponds, almost all eels differentiate into males, making it difficult to obtain females from farmed eel stocks (Okamura *et al.* 2013). However, even while working with eels from wild populations from the same area, a high degree of variability is evident with respect to the gonadal response to hormone treatment (in the present work, ovulation occurred between the 15th and the 29th week). Wild female eels originating from other brackish areas of the North Adriatic Sea (Mordenti *et al.* 2012) ovulated between the 19th and the 30th week. Other studies (Pedersen 2003, 2004; Palstra *et al.* 2005; Palstra & van den Thillart 2009) that employed wild eels in their reproduction programmes have also been characterized by large variation in time-to-maturity. Okamura *et al.* (2013) proposed that these differences in time-to-maturation could reflect specific differences in the maturity state of female eels just before the first hormone injection; it is plausible that especially gonadotropin receptor abundance may be important in this context.

We observed that among zootechnical performance indicators, the BWI at DHP injection was highly variable (from 108% to 134%) and it did not appear to be an adequate predictor of the final phase of ovarian maturation, as also reported by Palstra *et al.* (2005) and Mordenti *et al.* (2012), and for *A. japonica* by many authors (Seoka, Yamada, Iwata, Yanagisawa, Nakagawa & Kumai 2003; Dou *et al.* 2008; Chai *et al.* 2010; Ijiri *et al.* 2011; Unuma *et al.* 2011; Unuma, Sawaguchi, Hasegawa, Tsuda, Tanaka, Nomura & Tanaka 2012). The lack of a correlation between the BWI and the percentage of FTOs in the gonads in our study further reinforces the unsuitability of the BWI as a predictor of the right time for induction of final maturation in the European eel. Having said that, our findings do not concur with those from Palstra *et al.* (2005), who showed a relationship between the number of oocytes with single fat droplets (over-ripe and not fertilizable oocytes) and higher BWIs.

Ovarian biopsies obtained after the booster injection (the additional CPE administration following regular weekly injections) showed good synchronous development of oocytes, evidenced by 50% of oocytes being fully transparent. The stage synchrony of oocytes probably results from the low dosage of hormone initially administered to the eels (Mordenti *et al.* 2013). Synchronous maturation in the final phase is very important as it enhances the quantity of spawned eggs on the one hand and prevents the obstruction of the vent by immature ovarian tissue on the other; it is not coincidental that in this study the animals that provided the best yields (in terms of spawned eggs per %BW) had the highest percentage of FTOs. Furthermore, the use of high-dose CPE injections (40 mg kg^{-1} BW) from the 10th week onwards led to an acceleration in the final maturation phase, as observed by Chai *et al.* (2010) in *A. japonica*, and probably to a better quality of the eggs in terms of fertilization rate. However, there is a risk that migratory nucleus-stage oocytes develop too fast after the booster injection with CPE, and that a high proportion of oocytes contains a single fat droplet (over-ripe) prior to treatment with DHP (Chai *et al.* 2010). Unfortunately, over-ripening in eels progresses faster than in other teleosts (Ohta *et al.* 1996; Unuma, Kondo, Tanaka, Kagawa, Nomura & Ohta 2005).

Inappropriate timing of hormone administrations to pre-spawning eel broodstock may be a cause of poor egg quality. Unuma *et al.* (2011) suggested that proper timing of ovulation induction is essential, but that additional factors are also important for the acquisition of good quality eggs. To predict the best time of ovulation induction, the developmental stages of the oocytes in the ovary during final maturation should be evaluated. This study highlights that the best results in terms of spawned egg quantity and fertilization rate can be obtained when the proportion of FTO is at least 50% at the time of DHP injection. Indeed, it seems likely that the recent promising findings on spontaneous spawning of eels (Mordenti *et al.* 2014) carried out by our team may be due to fine-tuning of the timing of DHP administration.

This study convincingly illustrates that in our hands, and with the maturity criteria that we have employed, the spontaneous-spawning method produces higher quality eggs than what can be obtained by the stripping-insemination method, probably because the timing of spawning and fertilization is optimized by parent eels themselves (Okamura *et al.* 2013). In addition, the high incidence of spontaneous spawning in European eels in captivity was striking. The presence of good quality males in the tank is a decisive factor for spawning success: only a few minutes after a female was placed in the tank, typical mating behaviour was observed in males (approaching the head and urogenital region of the female; van Ginneken, Vianen, Muusze, Palstra, Verschoor, Lugten, Onderwater, van Schie, Niemantsverdriet, van Heeswijk, Eding & van den Thillart 2005). In sharp contrast, the only two females with unfertilized eggs (SPT-3 and SPT-7) were not approached by males displaying courtship behaviour. The better performance of the SPT-group compared with the STR-group is also reflected in the higher fertilization rate in the former group: for example, among the floating eggs in the SPT-eels, five of six females had a fertilization rate exceeding 70%. Retrieving floating eggs from all spawned females contrasts notably with the report on *A. japonica* by Seoka *et al.* (2003), in which floating eggs were obtained from four of 31 females. The buoyancy of eggs, important for oceanic survival and dispersal as well as for the initiation of early embryogenesis (Kagawa, Kishi, Gen, Kazeto, Tosaka, Matsubara, Matsubara & Sawaguchi 2011), has often been used as an indicator in the assessment of egg quality (Unuma *et al.* 2005). While buoyancy is not universally indicative of egg quality in teleost fish (e.g. Kohn & Symonds 2012), the ratio of floating eggs to total spawned eggs at least correlates positively with egg hatchability in eels (Unuma *et al.* 2005).

Conclusion

The present work shows that in *A. anguilla* the spontaneous-spawning method can yield better egg production, both in terms of quality and quantity, than the stripping-insemination method. Furthermore, BWI is not itself a proper predictor to guide the timing of DHP injection; rather, the presence of a high proportion of fully transparent oocytes can be used to time DHP injection to obtain eggs of good fertility.

Acknowledgments

This study was supported by Emilia-Romagna Region, Italy. Authors would like to thank INNOVAQUA srl (Reggio Emilia, Italy) for technical support. No conflict of interest has been identified.

References

Burgerhout E., Brittijn S.A., Kurwie T., Decker P., Dirks R.P., Palstra A.P., Spaink H.P. & van den Thillart G.E.E.J.M. (2011) First artificial hybrid of the eel species *Anguilla australis* and *Anguilla anguilla*. *BMC Developmental Biology* **11**, 16.

Casselman J.M. (2003) Dynamics of resources of American eel, *Anguilla rostrata*: declining abundance in 1990s. In: *Eel Biology* (ed. by K. Aida, K. Tsukamoto & K. Yamauchi), pp. 255–274. Springer-Verlag, Tokyo, Japan.

Chai Y., Tosaka R., Abe T., Sago K., Sago Y., Hatanaka E., Ijiri S. & Adachi S. (2010) The relationship between the developmental stage of oocytes in various season and the quality of the egg obtained by artificial maturation in the feminized Japanese eel *Anguilla japonica*. *Aquaculture Science* **58**, 269–278.

Dekker W. (2003) Status of the European eel stock and fisheries. In: *Eel Biology* (ed. by K. Aida, K. Tsukamoto & K. Yamauchi), pp. 237–254. Springer-Verlag, Tokyo, Japan.

Di Biase A., Bastone G., Casalini A., Parmeggiani A., Costantini G. & Mordenti O. (2012) Study of the morpho-physiological characteristics of two *Anguilla anguilla* populations selected for artificial reproduction. Word Aquaculture Society, Conference: "Global aquaculture:

securing our future - AQUA 2012" 1-5 September 2012, Prague, Czech Republic, 127–128.

Dou S.Z., Yamada Y., Okamura A., Shinoda A., Tanaka S. & Tsukamoto K. (2008) Temperature influence on the spawning performance of artificially-matured Japanese eel, *Anguilla japonica*, in captivity. *Environmental Biology of Fishes* **82**, 151–164.

Durif C.M.F., Dufour S. & Elie P. (2005) The silvering process of *Anguilla anguilla*: a new classification from the yellow resident to the silver migrating stage. *Journal of Fish Biology* **66**, 1025–1043.

Durif C.M.F., Dufour S. & Elie P. (2006) Impact of silvering stage, age, body size and condition on the reproductive potential of the European eel. *Marine Ecology Progress Series* **327**, 171–181.

EELREP (2006) Estimation of the reproduction capacity of European eel. Final report, EU Project, EELREP, Q5RS-2001-01836. Leiden University. Available at: http://www.fishbiology.net/EELREP_final_report.pdf. (accessed 15 June 2010)

Freyhof J. & Kottelat M. (2010) *Anguilla anguilla*. IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. Available at: www.iucnredlist.org (downloaded 8 January 2014).

Ijiri S., Tsukamoto K., Chow S., Kurogi H., Adachi S. & Tanaka H. (2011) Controlled reproduction in the Japanese eel (*Anguilla japonica*), past and present. *Aquaculture Europe* **36**, 13–17.

Kagawa H., Tanaka H., Ohta H., Okuzawa K. & Hirose K. (1995) In vitro effects of 17 alpha -hydroxyprogesterone and 17 alpha, 20 beta -dihydroxy-4-pregnen-3-one on final maturation of oocytes at various developmental stages in artificially matured Japanese eel *Anguilla japonica*. *Fisheries Science* **61**, 1012–1015.

Kagawa H., Kishi T., Gen K., Kazeto Y., Tosaka R., Matsubara H., Matsubara T. & Sawaguchi S. (2011) Expression and localization of aquaporin 1b during oocyte development in the Japanese eel (*Anguilla japonica*). *Reproductive Biology and Endocrinology* **9**, 71.

Kohn Y.Y. & Symonds J.E. (2012) Evaluation of egg quality parameters as predictors of hatching success and early larval survival in hapuku (*Polyprion oxygeneios*). *Aquaculture* **342–343**, 42–47.

Mordenti O., Di Biase A., Sirri R., Modugno S. & Tasselli A. (2012) Induction of sexual maturation in wild female European eel (*Anguilla anguilla*) in darkness and light conditions. *The Israeli Journal of Aquaculture - Bamidgeh*, **IJA_64.2012.726**, 9.

Mordenti O., Di Biase A., Bastone G., Sirri R., Zaccaroni A. & Parmeggiani A. (2013) Controlled reproduction in the wild European eel (*Anguilla anguilla*): two populations compared. *Aquaculture International* **21**, 1045–1063.

Mordenti O., Casalini A., Mandelli M. & Di Biase A. (2014) A closed recirculating aquaculture system for artificial seed production of the European eel (*Anguilla anguilla*): technology development for spontaneous spawning and egg incubation. *Aquacultural Engineering* **58**, 88–94.

Ohta H., Kagawa H., Tanaka H., Okuzawa K. & Hirose K. (1996) Changes in fertilization and hatching rates with time after ovulation induced by 17, 20β-dihydroxy-4- pregnen-3-one in the Japanese eel, *Anguilla japonica*. *Aquaculture* **139**, 291–301.

Ohta H., Kagawa H., Tanaka H., Okuzawa K. & Iinuma N. (1997) Artificial induction of maturation and fertilization in the Japanese eel, *Anguilla japonica*. *Fish Physiology and Biochemistry* **17**, 163–169.

Okamura A., Horie N., Mikawa N., Yamada Y. & Tsukamoto K. (2013) Recent advances in artificial production of glass eels for conservation of anguillid eel populations. *Ecology of Freshwater Fish* **23**, 95–110.

Oliveira K. & Hable W.E. (2010) Artificial maturation, fertilization and early development of the American eel, *Anguilla rostrata*. *Canadian Journal of Zoology* **88**, 1121–1128.

Palstra A.P. & van den Thillart G.E.E.J.M. (2009) Artificial maturation and reproduction of the European eel. In: *Spawning Migration of the European Eel* (ed. by G.E.E.J.M. van den Thillart, S. Dufour & C. Rankin), pp. 309–332. Springer, New York, USA.

Palstra A.P., Cohen E.G.H., Niemantsverdriet P.R.W., van Ginneken V.J.T. & van den Thillart G.E.E.J.M. (2005) Artificial maturation and reproduction of European silver eel: development of oocytes during final maturation. *Aquaculture* **249**, 533–547.

Pedersen B.H. (2003) Induced sexual maturation of the European eel *Anguilla anguilla* and fertilization of the eggs. *Aquaculture* **224**, 323–338.

Pedersen B.H. (2004) Fertilization of eggs, rate of embryonic development and hatching following induced maturation of the European eel *Anguilla anguilla*. *Aquaculture* **237**, 461–473.

Seoka M., Yamada S., Iwata Y., Yanagisawa T., Nakagawa T. & Kumai H. (2003) Differences in the biochemical content of buoyant and non-buoyant eggs of the Japanese eel, *Anguilla japonica*. *Aquaculture* **216**, 355–362.

Tatsukawa K. (2003) Eel resources in East Asia. In: *Eel Biology* (ed. by K. Aida, K. Tsukamoto & K. Yamauchi), pp. 293–298. Springer-Verlag, Tokyo, Japan.

Unuma T., Kondo S., Tanaka H., Kagawa H., Nomura K. & Ohta H. (2005) Relationship between egg specific gravity and egg quality in the Japanese eel, *Anguilla japonica*. *Aquaculture* **246**, 493–500.

Unuma T., Hasegawa N., Sawaguchi S., Tanaka T., Matsubara T., Nomura K. & Tanaka H. (2011) Fusion of lipid droplets in Japanese eel oocyte: stage classification and its use as a biomarker for induction of final oocyte maturation and ovulation. *Aquaculture* **322–323**, 142–148.

Unuma T., Sawaguchi S., Hasegawa N., Tsuda N., Tanaka T., Nomura K. & Tanaka H. (2012) Optimum

temperature of rearing water during artificial induction of ovulation in Japanese eel. *Aquaculture* **358–359**, 216–223.

van Ginneken V.J.T. & Maes G.E. (2005) The European eel (*Anguilla anguilla*), its lifecycle, evolution and reproduction: a literature review. *Reviews in Fish Biology and Fisheries* **15**, 367–398.

van Ginneken V.J.T., Vianen G., Muusze B., Palstra A.P., Verschoor L., Lugten O., Onderwater M., van Schie S., Niemantsverdriet P., van Heeswijk R., Eding E.P. & van den Thillart G.E.E.J.M. (2005) Gonad development and spawning behaviour of artificially-matured European eel (*Anguilla anguilla*). *Animal Biology* **55**, 203–218.