Innovatieve reproductie Europese aal

VIP report

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Contents

Samenvatting

Binnen VIP project "Innovatieve Reproductie van Europese Aal' ("Perspectief voor een duurzame visserij" #4610010911889 aan R.P. Dirks, NewCatch) was contractpartij IMARES verantwoordelijk voor experimenteel onderzoek naar conditionering van de opgekweekte alen tot schieralen met hoge reproductiecapaciteit; maturatieprotocollen onder fotothermale stimulatie en het testen van larvale dieten, om daarmee bij te dragen aan twee doelstellingen van het project:

1: Het onderzoeken of natuurlijke prikkels, zoals fotothermale stimulering en zoutwaterprikkels, gebruikt kunnen worden voor het verhogen van de efficiëntie van het maturatie-protocol.

2: Het gericht zoeken naar geschikte voeding voor vroege palinglarven met behulp van beschikbare kennis met betrekking tot de genetische code en microbiële constitutie van het maagdarm-stelsel van de Europese aal.

Daartoe zijn twee grootschalige experimenten uitgevoerd*:

In het eerste experiment zijn relatief oude mannelijke en vrouwelijke kweekalen op de kwekerij voor 6 maanden gevoerd met een commercieel broodstock dieet, vervolgens onderworpen aan een gesimuleerde zoet- en zoutwatermigratie en uiteindelijk door hormonale stimulering tot seksuele maturatie en reproductie gebracht.

In het tweede experiment zijn gefeminiseerde alen van NewCatch voor 6 maanden gevoerd met makreelfilets en samen met een tweede batch gefeminiseerde alen van NewCatch en mannelijke kweekalen onderworpen aan een identieke gesimuleerde zoet- en zoutwatermigratie als in experiment 1. Vervolgens is een deel van deze geconditioneerde gefeminiseerde alen naar Glasaal Volendam getransporteerd voor hormonale stimulering en reproductie en een ander deel is bij IMARES door hormonale stimulering tot seksuele maturatie en reproductie gebracht.

Door gesimuleerde migratie konden zowel mannelijke als vrouwelijke alen tot pubering worden aangezet. Toenamen in gonadenmassa en oogdiameters toonden aan dat de gesimuleerde migratie de vroege seksuele maturatie stimuleerde. Daarmee kan gesimuleerde migratie worden gebruikt om kweekalen schier te maken en in de juiste staat te brengen voor reproductie experimenten. Deze resultaten zijn gepubliceerd in een paper in Aquaculture. Gefeminiseerde alen bleken nog gevoeliger voor de behandeling en oogdiameters namen nog sterker toe.

Zowel dieren van experiment 1 als experiment 2 zijn door hormonale stimulatie tot volledige maturatie en reproductie aangezet teneinde larven te produceren en in leven te houden tot het moment van exogeen voeden. In experiment 1 matureerde 79% van de vrouwelijke alen (GSI>30) en werden batches van 5 vrouwelijke alen bevrucht. In experiment 2 matureerde 21% van de gefeminiseerde alen en werden batches van 4 vrouwelijke alen bevrucht. Vroege celdelingen waren meestal asymmetrisch en in alle gevallen werd 24 uur na bevruchting geen verdere ontwikkeling meer geobserveerd. Door het ontbreken van voedende larven konden voeders niet experimenteel worden getest.

* hiertoe is ook bijgedragen vanuit Kennisbasis Onderzoek (KB).

Ia. Gesimuleerde migratie van oude kweekalen

Simulated migration under mimicked photothermal conditions enhances sexual maturation of farmed European eel (*Anguilla anguilla*)

1. Introduction

Puberty in fish is the developmental period during which fish become capable of reproducing sexually for the first time (Taranger et al., 2010). As the numerous developmental changes that occur during eel silvering are primarily induced by steroids, silvering can be considered as eel's early puberty (Rousseau et al., 2009). At the start of early puberty, European eels (*Anguilla anguilla* Linnaeus 1758) leave their freshwater habitat to undertake an approximate 6,000 km reproductive migration to the spawning area in the Sargasso sea (Schmidt, 1923). When eels are prevented from undertaking their reproductive migration, sexual maturation remains dually blocked by a deficiency of gonadotropin releasing hormone (GnRH) and inhibition by dopamine (Vidal et al., 2004; Weltzien et al., 2009) within the brain-pituitary-gonad (BPG) axis. This blockage represses the synthesis and release of the pituitary gonadotropins (GTHs): luteinizing hormone (LH) and follicle-stimulating hormone (FSH) preventing the gonads to develop further (Weltzien et al., 2009).

The arrested development of sexual maturation in pre-migrant eels occurs early in puberty and impedes the reproduction of European eel in captivity. Numerous studies have applied hormonal injection protocols with pituitary extracts to artificially induce sexual maturation but thus far these trials have only yielded low gamete quality and limited reproductive success (reviewed by Palstra and van den Thillart, 2009). Although currently, larvae can be produced regularly and kept alive until exogenous feeding, low gamete quality remains a major issue (Sørensen et al., 2014; Tomkiewicz et al. 2012). One possible explanation for the low gamete quality is that the oocytes of pre-migrating silver eels are still too premature to be optimally stimulated in their development with pituitary extracts of spawning carp or salmon. This is particularly true for farmed silver eels as they still have low GSI values in comparison with wild conspecifics that are caught at the final stages of their downstream migration, just before oceanic migration (Durif et al. 2009). Therefore, to produce high quality gametes, we hypothesize that it will be essential to design broodstock conditioning protocols that maximally enhance sexual maturation (puberty) by subjecting farmed silver eels to natural factors. By using natural factors to promote sexual maturation, artificial induction of sexual maturation through hormonal injections may be minimized and gamete quality improved.

Several parameters may affect the progress of sexual maturation during reproductive migration in *A. anguilla*, including the performance of sustained exercise and (daily fluctuations in) environmental conditions such as salinity, temperature, light and pressure. Swimming exercise of female silver eels in fresh water (FW) promotes the deposition of lipids during early oocyte development, but swimming in seawater (SW) suppresses further maturation (Palstra and van den Thillart, 2010; Palstra et al., 2009, 2007). In wild male eels, in contrast, SW-exercise increases gonad weight and induces spermatogenesis (Palstra et al., 2008). Sexual maturation can be positively affected by an increase in salinity as experienced during the change from fresh water to seawater (as shown for female Japanese eel (*Anguilla japonica*) by Kagawa, 2003; Kagawa et al., 1998). Photothermal control concerns the regulatory impact of temperature and day length which can be considered as a trigger in synchronising sexual maturation events (Kjesbu et al., 2010). European eels migrating in fresh water in autumn experience decreasing light and temperature conditions which appear to advance sexual maturation (respectively Boëtius and Boëtius, 1967; Mordenti et al., 2012 and Pérez et al., 2011). During oceanic migration, eels experience fluctuations in temperature during daily vertical migration (Aarestrup et al., 2009).

In this study we tested whether, and to which degree, sexual maturation in farmed European silver eels is advanced by simulated migrations in FW and SW under mimicked photothermal conditions.
As effects of individual factors on the sexual maturation in European eel have been studied and are mostly known, in this study we apply a multifactorial approach combining factors in treatments with two different settings of these factors corresponding to the two treatments mimicking FW and SW migration. This approach of simulating migration in the lab with samplings at different time-points is similar to studies with samplings at different locations along the migration route where the combinations of factors as experienced during that part of the migration route also act as the experimental treatment (Miller et al., 2009; Onuma et al, 2009ab, 2010; Ueda, 2011). Applying a multifactorial approach to study effects is well accepted in toxicological and medical studies studying the effects of cocktails of drugs, antibodies or contaminants as experimental treatments (e.g. respect. Zhou et al., 2004; Logtenberg, 2007; Celander, 2011 and also van Ginneken et al., 2009). Moreover, this approach represents a necessary

step from studying the effects of single factors towards application that directly benefits commercial aquaculture. This knowledge can be applied to enhance gamete quality in reproduction protocols by minimizing the artificial component consisting of hormonal injections, and/or to increase the sensitivity of pre-treated eels to subsequent hormonal injections.

2. Materials and Methods

Experimental protocols complied with the current laws of the Netherlands and were approved by the animal experimental committee (DEC nr. 2013163).

2.1 Experimental animals and setup

Farmed male (n=186) and female (n=121) silver eels were obtained from a commercial eel farm (Passie voor Vis, Sevenum, the Netherlands). They came from a single tank with male and female eels that had been kept after reaching market size at a density of 180 kg m^{-3}. Males were 3 years old, females originated from different juvenile batches and were 4-5 years old. They were reared in FW at 23.5 °C under a 24L:0D photoperiod. Eels were selected for sex by scoping out either large females or small males. At IMARES' facilities, ten males and ten females were selected one by one, by netting several and then releasing all except for one, and placed in a 250 L circular control tank, which was part of a 425 L RAS unit ('START' group; Table 1). All remaining eels were introduced in a 3,600 L Brett-type swim flume (Brett, 1964) as described by Palstra et al. (2015; Fig. 1) to acclimatize for three days. After acclimatization, eels in the flume were PIT-tagged (Trovan, DorsetID, Aalten, the Netherlands) under anaesthesia. All eels from the START group were sampled and males (n=10) and females (n=10) were selected and transferred from the swim flume to the control tank to serve as control group ("C1"; 23.5 °C; 0‰; 24L:0D; Table 1) for the eels in the swim flume. These eels were subjected to a simulation of FW migration under natural photothermal conditions (12.0 ± 0.0 °C; 0‰; 8L:16D; Table 1) and at a flow of 0.57 m s^{-1}, which is the optimal swimming speed for group-wise migrating male *A. anguilla* (Burgerhout et al., 2013b) and which corresponded to 1.34 Body Length per second (BL s^{-1}) for the experimental males and 0.79 BL s^{-1} for the females in this study. After 14 days, migrating eels had swum 689 km which corresponds to maximal migration distances as experienced in the wild and 10 males and 10 females were sampled as "M1" group, as well as the 20 eels from the control tank as the "C1" group (Table 1). From the remaining eels in the flume, selected males (n=10) and females (n=10) were transferred to the control tank to serve as a control group (11.5 °C, 0‰, 8L:16D; Table 1) for the eels in the flume, which were subjected to a simulated seawater migration ("M2") in complete darkness, again at a flow of 0.57 m s^{-1}. Salinity in the swimming flume was gradually increased by replacing water with natural seawater over three days (day 1: 50%; day 2: 75% and day 3: 100%) to 29.2 ± 0.0‰ and temperature was fluctuated between 11.7 °C for 8 h day^{-1} (0:00 h to 8.00 h) and 10.1 °C for 16 h day^{-1} (8:00 h to 0:00 h; Fig. 2) to simulate the average temperature profile that is experienced during the daily vertical migrations in the ocean (Aarestrup et al., 2009). Water in the flume took 3h17m ± 3m to heat from 10.1 °C to 11.7 °C, and 3h3m ± 2m vice versa. After 63 days of seawater migration (total distance swum during SW migration: 3,103 km) which should reveal any significant effects of this treatment on sexual maturation, 10 males and 10 females were selected and sampled as "M2" group, as well as the 20 control eels from the control tank as "C2" group (Table 1). Eels were not fed during the entire experiment.

2.2 Sampling and biometrics

Per sampling event, ten males and ten females per treatment were anaesthetized and blood was extracted from the caudal vein using heparin flushed syringes on ice. Haematocrit (Hct) values were measured in 9 µl blood samples as triplicates per eel. Hct is a first indicator of the physical condition and represents a factor that (cor)relates negatively with the progression of sexual maturation in females. The remaining blood was centrifuged (5 min; 9,500 × g; 4 °C) and blood plasma was stored at -80 °C until later hormone measurements. Total body length (BL), body weight (BW), body girth (BG), pectoral fin length (PFL), gonad weight (GW), liver weight (LW) and horizontal and vertical eye diameter (EDh and EDv, respectively) were measured. The following indices were calculated: Fulton's condition factor (K; Palstra et al., 2011), body girth index (BGI; Palstra and van den Thillart, 2009), pectoral fin length index (PFLI; Palstra et al., 2011), gonadosomatic index (GSI; Palstra et al., 2011), hepatosomatic index (HSI; Palstra et al., 2011) and eye index (EI; Pankhurst, 1982).

2.3 Plasma analyses

Testosterone (T) plasma levels were measured in undiluted plasma as duplicates using commercial enzyme immunoassay (EIA) testosterone kits (Cayman Chemical, Ann Arbor, USA). Absorbance was measured at 405 nm and the detection limit was between 6.5 and 9.0 pg ml^{-1}. Gonadotropin (LH and FSH) levels were determined in undiluted blood plasma as duplicates using a recently developed eel gonadotropin receptor-based bioassay (Minegishi et al., 2012). Trypsinized cells were incubated in the 96-well plates for 48 h to ensure proper adhesion of the cells to the well plates. Gonadotropin levels in all plasma samples were too low to fit the linear range of the standard curve. Therefore, GTH levels were expressed as fluorescence counts per second (CPS) as measured in the fluorometric bioassay, which provided the relative differences between groups but not the absolute GTH concentrations. LH and FSH were determined in separate hormone-specific bioassays.

2.4 Statistics

Only BL and BW data of female eels were found to be normally distributed (Shapiro-Wilk tests). Therefore, female BL and BW data were pair-wise compared between treatments with two-tailed student's t-tests. All other data were compared between treatments using non-parametric Mann-Whitney U tests. BL, BW, K and BGI data were tested two-tailed while all other data were tested one-tailed for progression of sexual maturation. Data were compared within sex and only the following useful pairwise comparisons were made: C1 *vs.* START, M1 *vs.* C1, C2 *vs.* M1 and M2 *vs.* C2. All values are expressed as average ± standard error. Differences were considered significant at $P<0.05$.

3. Results

3.1 Swimming behaviour

Eels in the swimming flume aggregated in a school and displayed group-wise positive rheotactic swimming behaviour. No apparent signs of stress or disease were observed during the simulated migration and no mortality occurred as an effect of treatment.

3.2 Simulated freshwater migration

After the two-week simulated freshwater migration (M1), male eels showed significantly (> 4-fold) elevated plasma testosterone and a higher HSI as compared to control eels (C1), and haematocrit was 22% lower (Table 2). HSI was significantly lower in the controls as compared to the START group, indicating a time/starvation effect. Both GW and GSI were higher in the controls *vs.* eels of the START group. Plasma gonadotropin levels remained near the detection limits of the assays and male plasma LH was elevated in the controls as compared to the START group but no differences were found as a result of simulated migration. Plasma FSH levels were not elevated in any treatment or control group.

Similar as with males, female freshwater migrants (M1) showed significantly higher (> 2-fold) plasma T levels, higher HSI and 10% lower haematocrit (Table 3) as compared to controls (C1). Female GW and GSI were not elevated in the controls nor in the treatment group. Also in females, plasma gonadotropin levels remained near the detection limits of the assays and were not different between female migrants and controls. Comparable to males, female controls showed elevated plasma LH levels as compared to eels of the START group. Similar as with males, plasma FSH levels were also not elevated in any treatment or control group.

3.3 Simulated seawater migration

Male seawater migrants (M2) showed increased GW, GSI, EI and plasma T levels *vs.* the controls (C2; Table 2). Both GW and GSI were significantly lower in the controls compared to eels of the M1 group, as was LH.

Female GSI was significantly higher in SW-migrants (M2; 1.40 ± 0.06) as compared to the controls (C2) and this was also apparent for GW (Table 3). Female EI increased during the course of the experiment from 9.7 ± 0.5 (START) to 11.5 ± 2.1 (M2) but without differences between migrating and control eels.

4. Discussion

There is a high urgency to close the life cycle of European eel in captivity in order to establish a self-sustaining aquaculture that can operate independently from the glass eel arrivals in nature. Because fishing on migratory silver eels has been prohibited in national eel management protocols in most European countries (ICES, 2012), farmed silver eels should be considered as broodstock. However, farmed silver eels are less mature than wild silver eels that are about to start their oceanic migration (respectively, male GSI: 0.026% vs. ~0.10% and female GSI: 0.70% vs. ~1.50%; this study vs. data from Palstra et al., 2008). Sexual maturation of farmed silver eels should therefore be advanced to bring these eels to a similar maturity level as wild migrant eels. In this study, farmed silver eels were subjected to anorexic, mixed-sex, group wise freshwater and seawater migrations under mimicked photothermal conditions to enhance their sexual maturation. Our results show that a 3,792 km simulated reproductive migration promotes steroid-regulated silvering and sexual maturation in both male and female silver eels.

This is the first experimental study to investigate the combined effects of the natural factors photoperiod, temperature, salinity and exercise on the sexual maturation of eel. Photoperiod is a pivotal environmental factor in cueing and timing the reproduction of many teleosts (e.g. Andersson et al., 2013; Bromage et al., 2001; Palstra et al., 2015), but detailed information concerning the effects of photoperiod on the BPG axis in silver eels is lacking. Low temperature reduced pituitary gonadotropin expression and did not increase GSI in farmed female *A. japonica*, although oocyte diameter was increased and oil droplet formation in the oocytes was promoted (Sudo et al., 2011). The change in salinity when replacing the fresh water with seawater seems an important factor to have contributed to the observed stimulation of sexual maturation, because significant increases in GSI have previously been observed in female farmed *A. japonica* after a three-month rearing period in seawater (Kagawa et al. 1998; Kagawa, 2003). Sustained exercise is known to advance sexual maturation and induce spermatogenesis in wild males (Palstra et al., 2008), but this effect was not observed when exercising farmed males (Burgerhout et al., 2013a). Our study showed that the sexual maturation of farmed eels is advanced by the integrated effects of exercise, salinity and the natural photothermal regime.

Eels swim at a depth of 200 m during night time and then dive to depths of 600 m to over 1000 m (Aarestrup et al., 2009). At depths below 200 m, light intensity falls to 1% of the value at the surface. Therefore we have performed the experiment in darkness. Still, diel vertical migration suggests that migrating eels do sense differences in light intensity at these depths. A very recent paper (Chow et al., 2015) shows there is a positive correlation between migration depth and light intensity indicating that the eels were sensitive to sunlight and to moonlight. This would also provide a functional explanation for the enlargement of the eyes and the structural changes that occur during silvering as adaptations to increase this sensitivity in order to perform diel vertical migration and to navigate. Still, these are correlations and observed in a low number of eels. The mechanism behind an increase in light sensitivity and how that relates to diel vertical migration, navigation and progressing sexual maturation deserves more research efforts. The high pressures as experienced when swimming at great depths are known to affect sexual maturation (Sébert et al., 2007) but could not be simulated in this experimental set-up.

The simulated freshwater and seawater migrations stimulated the progression of sexual maturation similarly for both sexes (Table 1, 2). Simulated freshwater migration increased plasma T levels in males and females. The observed increased HSI and decreased Hct values in freshwater migrants are likely metabolic effects, induced through starvation (Caruso et al., 2010; Johansson-Sjöbeck et al., 1975; Larson and Lewander, 1973) and perhaps reinforced by exercise, and/or reduced temperature (Gollock et al., 2005; Laursen et al., 1985). Simulated seawater migration enhanced gonad development (significantly increased GW and GSI) for both sexes. In contrast to the females, migratory males showed an increased EI, which is associated with sex steroid production and gonadal development and is therefore an external indicator for sexual maturation (Pankhurst, 1982). Indeed, plasma T levels were also higher in males after simulated SW-migration. The GSI values of the male migrants were approaching the GSI values of their wild conspecifics (Palstra et al., 2008) and the EI was even higher (Durif et al., 2005). Stimulation of gonadal development in females was achieved up to levels corresponding to their wild counterparts that are ready to embark on their oceanic migration.

The absence of higher gonadotropin plasma levels in our study indicates that the processes which are under gonadotropin control, such as vitellogenesis, were not yet triggered. European eels with GSI values < 2 generally do not contain oocytes with yolk globuli (Palstra et al., 2007, 2010ab). The

observed induced progression in sexual maturation appeared to be under steroid control, as plasma T levels were elevated in experimental males and females. As gonadotropin secretion by the pituitary is stimulated by GnRH and inhibited by dopamine (Dufour et al., 1983; 1985; Mylonas and Zohar, 2007), the eels in our study may still remain under dopaminergic inhibition (Dufour et al., 2005). The question therefore remains which environmental trigger will be pivotal in lifting dopaminergic inhibition and consequently initiating vitellogenesis. This question may best be answered for other *Anguilla* species than *Anguilla anguilla*: species that swim a shorter distance to the spawning grounds and are already in a vitellogenic state when embarking on their oceanic migration (Todd, 1981; Burgerhout et al., 2011).

This study has demonstrated that a simulated reproductive migration under natural photothermal conditions advances sexual maturation in farmed silver eels, and produces farmed eel broodstock in a stage of silvering which is comparable to that of wild eel. Conditioning through simulated migration promoted the steroid-regulated component of silvering; more advanced gonadotropin-regulated sexual maturation was not induced. Until natural triggers that promote a more significant secretion of gonadotropins in European eel are eluded, hormonal protocols administering gonadotropins remain pivotal in stimulating vitellogenic events and final maturation. The knowledge from the current study may be applied to make farmed eels more silver and therewith improve the susceptibility of farmed eel broodstock to hormonal stimulation.

This study has also demonstrated that a simulated reproductive migration under natural photothermal conditions does not fully mature farmed silver eels, neither males nor females. Although it is tempting to extrapolate this result to the natural situation and draw the conclusion that European eels will not reach advanced vitellogenic stages before reaching the Sargasso sea, earlier results (Palstra et al., 2008 vs. Burgerhout et al., 2013a) have shown that we need to be cautious in drawing such conclusions about eels in the wild when using farmed eels. In order to obtain insights about the progress of maturation during oceanic migration, a similar experiment should be repeated using migrating silver eels from the wild.

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6. References

Aarestrup, K., Økland, F., Hansen, M.M., Righton, D., Gargan, P., Castonguay, M., Bernatchez, L., Howey, P., Sparholt, H., Pedersen, M.I., McKinley, R.S., 2009. Oceanic spawning migration of the European eel (*Anguilla anguilla*). Science. 325, 1660.

Andersson, E., Schulz, R.W., Male, R., Bogerd, J., Patiña, D., Benedet, S., Norberg, B., Taranger, G.L., 2013. Pituitary gonadotropin and ovarian gonadotropin receptor transcript levels: Seasonal and photoperiod-induced changes in the reproductive physiology of Atlantic salmon (*Salmo salar*). Gen. Comp. Endocrin. 191, 247-258.

Boëtius, I., Boëtius, J., 1967. Studies in the European Eel, *Anguilla anguilla* (L.). Experimental induction of the male sexual cycle, its relation to temperature and other factors. Medd. Dan. Fish. Havunders. New. Ser. 4, 339-405.

Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Can. 21(5), 1183-1226.

Bromage, N., Porter, M., Randall, C., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture. 197, 63-98.

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

Burgerhout, E., Brittijn, S.A., Tudorache, C., de Wijze, D.L., Dirks, R.P., van den Thillart, G.E.E.J.M., 2013a. Male European eels are highly efficient long distance swimmers: effects of endurance swimming on maturation. Comp. Biochem. Physiol. A. 166, 522-527.

Burgerhout, E., Tudorache, C., Brittijn, S.A., Palstra, A.P., Dirks, R.P., van den Thillart, G.E.E.J.M., 2013b. Schooling reduces energy consumption in swimming male European eels, *Anguilla anguilla* L. J. Exp. Mar. Biol. Ecol. 448, 66-71.

Caruso, G., Maricchoiolo, G., Micale, V., Genovese, L., Caruso, R., Denaro, M.G., 2010. Physiological responses to starvation in the European eel (*Anguilla anguilla*): effects on haematological, biochemical, non-specific immune parameters and skin structures. Fish. Physiol. Biochem. 36, 71-83.
Celander, M.C., 2011. Cocktail effects on biomarker responses in fish. Aquat. Toxicol. 105S, 72– 77.
Chow, S., Okazaki, M., Watanabe, T., Segawa, K., Yamamoto, T., Kurogi, H., Tanaka, H., Ai, K., Kawai, M., Yamamoto, S., Mochioka, N., Manabe, R., Miyake, Y., 2015. Ligth-sensitive vertical migration of the Japanese eel Anguilla japonica revelaed by real-time tracking and its utilization for geolocation. PLoS ONE 10(4): e0121801.
Dufour, S., Delerue-La Belle, N., Fontaine, Y.A., 1983. Effects of steroid hormones on pituitary immunoreactive gonadotropin in European fresh-water eel, *Anguilla anguilla* L. Gen. Comp. Endocrinol. 52, 190-197.
Dufour, S., Fontaine, Y.A., Kerdelhue, B., 1985. Increase in brain and pituitary radioimmunuassayable gonadotropin releasing hormone (GnRH) in the European silver eel treated with sexual steroid or human chorionic gonadotropin. Neuropeptides. 6, 495-502.
Dufour, S., Weltzien, F-A., Sebert, M-E., Le Belle, N., Vidal, B., Vernier, P., Pasqualini, C., 2005. Dopaminergic inhibition of reproduction in teleost fishes: ecophysiological and evolutionary implications. Ann. N. Y. Acad. Sci. 1040, 9-21.
Durif, C., Dufour, S., Elie, P., 2005. The silvering process of *Anguilla anguilla*: a new classification from the yellow resident to the silver migrating stage. J. Fish. Biol. 66, 1025-1043.
Durif, M.F., van Ginneken, V., Dufour, S., Müller, T., Elie, P., 2009. Seasonal evolution and individual differences in silvering eels from different locations, in: van den Thillart, G., Dufour, S., Ranking, J.C. (Eds.), Spawning migration of the European eel. Springer, the Netherlands, pp. 13-38.
Gollock, M.J., Kennedy, C.R., Brown, J.A., 2005. Physiological responses to acute temperature increase in European eels *Anguilla anguilla* infected with *Anguillicola crassus*. Dis. Aquat. Org. 64, 223-228.
ICES, 2012. Report of the Joint EIFAAC/ICES Working Group on Eels (WGEEL), 3-9 September 2012, Copenhagen, Denmark. ICES CM 2012/ACOM:18. pp. 824
Johansson-Sjöbeck, M.-L., Dave, G., Larsson, Å., Lewander, K., Lidman, U., 1975. Metabolic and hematological effects of starvation in the European eel, *Anguilla anguilla* L.-II. Hematology. Comp. Chem. Physiol. A. 52, 431-434.
Kagawa, H., 2003. Artificial induction of oocyte maturation and ovulation, in: Aida, K., Tsukamoto, K., Yamauchi, K. (Eds.), Eel Biology. Springer, Tokyo, pp. 401-414.
Kagawa, H., Iinuma, N., Tanaka, H., Ohta, H., Okuzawa, K., 1998. Effects of rearing period in seawater on induced maturation in female Japanese eel *Anguilla japonica*. Fish. Sci. 64, 77-82.
Kjesbu, O.S., Righton, D., Krüger-Johnsen, M., Thorsen, A., Michalsen, K., Fonn, M., Witthames, P.R., 2010. Thermal dynamics of ovarian maturation in Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 67, 605-625.
Laursen, J.S., Andersen, N.A., Lykkeboe, G., 1985. Temperature acclimation and oxygen binding properties of blood of the European eel *Anguilla anguilla*. Comp. Biochem. Physiol. A. 81, 79-86.
Logtenberg, T., 2007. Antibody cocktails: next-generation biopharmaceuticals with improved potency. TRENDS Biotechnol. 25, 9.
Miller, K.M., Schulze, A.D., Ginther, N., Li, S., Patterson, D.A., Farrell, A.P., Hinch, S.G., 2009 Salmon spawning migration: metabolic shifts and environmental triggers. Comp. Biochem. Physiol. D. 4, 75–89.
Minegishi, Y., Dirks, R.P., de Wijze, D.L., Brittijn, S.A., Burgerhout, E., Spaink, H.P., van den Thillart, G.E.E.J.M., 2012. Quantitative bioassays for measuring biologically functional gonadotropins based on eel gonadotropic receptors. Gen. Comp. Endocr. 178, 145-152.
Mordenti, O., Di Biase, A., Sirri, R., Modugno, S., Tsseli, A., 2012. Induction of sexual maturation in wild female European eels (*Anguilla anguilla*) in darkness and light. Isr. J. Aquac. Bamidgeh. 64, http://hdl.handle.net/10524/23179 (accessed on 10/13/2014).
Mylonas, C.C., Zohar, Y., 2007. Promoting oocyte maturation, ovulation and spawning in farmed fish, in: Babin, P.J., Cerdà, J., Lubzens, E. (Eds.), The fish oocyte: from basic studies to biotechnological applications. Springer, the Netherlands, pp. 437-474.
Onuma, T.A., Sato, S., Katsumata, H., Makino, K., Hu, W.W., Jodo, A., Davis, N.D., Dickey, J.T., Ban, M., Ando, H., Fukuwaka, M., Azumaya, T., Swanson, P., Urano, A., 2009. Activity of the pituitary-gonadal axis is increased prior to the onset of spawning migration of chum salmon. J. Exp. Biol. 212, 56–70.
Onuma, T.A., Makino, K., Ban, M., Ando, H., Fukuwaka, M., Azumaya, T., Swanson, P., Urano, A., 2009. Elevation of the plasma level of insulin-like growth factor-I with reproductive maturation prior to initiation of spawning migration of chum salmon. Ann. NY Acad. Sci. 1163, 497–500.
Onuma, T.A., Makino, K., Katsumata, H., Beckman, B.R., Ban, M., Ando, H., Fukuwaka, M., Azumaya, T., Swanson, P., Urano, A., 2010. Changes in the plasma levels of insulin-like growth factor-I from the onset of spawning migration through upstream migration in chum salmon. Gen. Comp. Endocrinol. 165, 237–243.
Palstra, A., van den Thillart, G., 2009. Artificial maturation and reproduction of the European eel, in: van den Thillart, G., Dufour, S., Rankin, J.C. (Eds.), Spawning migration of the European eel. Springer, Berlin, pp. 229-251.

Palstra, A.P., van den Thillart, G.E.E.J.M., 2010. Swimming physiology of European silver eels (*Anguilla anguilla* L.): energetic costs and effects on sexual maturation and reproduction. Fish. Physiol. Biochem. 36, 297-322.
Palstra, A., Curiel, D., Fekkes, M., de Bakker, M., Székely, C., van Ginneken, V., van den Thillart, G., 2007. Swimming stimulates oocyte development in European eel. Aquaculture. 270, 321-332.
Palstra, A.P., Schnabel, D., Nieveen, M.C., Spaink, H.P., van den Thillart, G.E.E.J.M., 2008. Male silver eels mature by swimming. BMC Physiol. 8:14.
Palstra, A., van Ginneken, V., van den Thillart, G., 2009. Effects of swimming on silvering and maturation of the European eel, *Anguilla anguilla* L, in: van den Thillart, G., Dufour, S., Rankin, J.C. (Eds.), Spawning migration of the European eel. Springer, Berlin, pp. 229-251.
Palstra, A.P., Schnabel, D., Nieveen, M.C., Spaink, H.P., van den Thillart, G.E.E.J.M., 2010a. Temporal expression of hepatic estrogen receptor 1, vitellogenin1 and vitellogenin2 in European silver eels. Gen. Comp. Endocrinol. 166, 1-11.
Palstra, A.P., Schnabel, D., Nieveen, M., Spaink, H., van den Thillart, G., 2010b. Swimming suppresses hepatic vitellogenesis in European silver eel as shown by quantitative RT-PCR of the estrogen receptor 1, vitellogenin1 and vitellogenin2 in the liver. Reprod. Biol. Endocrinol. 8: 27.
Palstra, A.P., Angeles Guerrero, M., de Laak, G., Klein Breteler, J.P.G., van den Thillart, G.E.E.J.M., 2011. Temporal progression in migratory status and sexual maturation in European silver eels during downstream migration. Fish. Physiol. Biochem. 37, 285-296.
Palstra, A.P., Mes, D., Kusters, K., Roques, J.A.C., Flik, G., Kloet, K., Blonk, R.J.W., 2015. Forced sustained swimming exercise at optimal speed enhances growth of juvenile yellowtail kingfish (*Seriola lalandi*). Front. Physiol. 5:506.
Palstra, A.P., Blok, M.C., Kals, J., Blom, E., Tuinhof-Koelma, N., Dirks, RP., Forlenza, M. , Blonk, R.J.W., 2015. In- and outdoor reproduction of first generation common sole *Solea solea* under a natural photothermal regime: temporal progression of sexual maturation assessed by monitoring plasma steroids and pituitary gonadotropin expression. Gen. Comp. Endocrin. SI: 10ISRPF, accepted.
Pankhurst, N.W., 1982. Relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguilla* (L.). J. Fish. Biol. 21, 127-140.
Pérez, L., Peñaranda, D.S., Dufour, S., Baloche, S., Palstra, A.P., van den Thillart, G.E.E.J.M., Asturiano, J.F., 2011. Influence of temperature regime on endocrine parameters and vitellogenesis during experimental maturation of European eel (*Anguilla anguilla*) females. Gen. Comp. Endocr. 174, 51-59.
Rousseau, K., Aroua, S., Schmitz, M., Elie, P., Dufour, S., 2009. Silvering: Metamorphosis or Puberty?, in: van den Thillart, G., Dufour, S., Rankin, J.C. (Eds.), Spawning migration of the European eel. Springer, Berlin, pp. 39-64.
Schmidt, J., 1923. Breeding places and migration of the eel. Nature. 111, 51-54.
Sébert, M-E., Amérand, A., Vettier, A., Weltzien, F-A, Pasqualini, C., Sébert, P., Dufour, S., 2007. Effects of high hydrostatic pressure on the pituitary–gonad axis in the European eel, *Anguilla anguilla* (L.). Gen. Comp. Endocrinol. 153, 289–298.
Sørensen, S. R., Skov, P. V., Lauesen, P., Tomkiewicz, J., Bossier, P., De Schryver, P., 2014. Microbial interference and potential control in culture of European eel (*Anguilla anguilla*) embryos and larvae. Aquaculture. 426-427, 1-8.
Sudo, R., Tosaka, R., Ijiri, S., Adachi, S., Suetake, H., Suzuki, Y., Horie, N., Tanaka, S., Aoyama, J., Tsukamoto, K., 2011. Effect of temperature decrease on oocyte development, sex steroids, and gonadotropin *β*-subunit mRNA expression levels in female Japanese eel *Anguilla japonica*. Fish. Sci. 77, 575-582.
Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F-A, Dufour, S., Karlsen, Ø., Norberg, B., Andersson, E., Hansen, T., 2010. Control of puberty in farmed fish. Gen. Comp. Endocrinol. 165, 483–515.
Todd, P.R., 1981. Timing and periodicity of migrating New Zealand freshwater eels (Anguilla spp,). New. Zeal. J. Mar. Fresh.15, 225-235.
Tomkiewicz, J., Tybjerg, L., Støttrup, J.G., McEvoy, F., Ravn, P., Sørensen, S.R., Lauesen, P., Graver, C., Munk, P., Holst, L.K., Vestbö, B., Svalastoga, E., Jacobsen, C., Holst, B., Steenfeldt, S.J., Hornum, T., Kofoed T., 2012. Reproduction of European eel in aquaculture (REEL). DTU Aqua Report Series (47 pp.).
Ueda H., 2011. Physiological mechanism of homing migration in Pacific salmon from behavioral to molecular biological approaches. Gen. Comp. Endocrinol. 170, 222–232.
van Ginneken, V., Palstra, A., Leonards, P., Nieveen, M., van den Berg, H., Flik, G., Spannings, T., Niemantsverdriet, P., van den Thillart, G., Murk, T. (2009) PCBs and the energy costs of migration in European silver eel (*Anguilla anguilla*). Aquat. Toxicol. 92, 213-220
Vidal, B., Pasqualini, C., Le Belle, N., Holland, M.C.H., Sbaihi, M., Vernier, P., Zohar, Y., Dufour, S., 2004. Dopamine inhibits luteinizing hormone synthesis and release in the juvenile Europeal eel: a neuroendocrine lock for the onset of puberty. Biol. Reprod. 71, 1491-1500.
Weltzien, F-A., Sébert, M-E., Vidal, B., Pasqualini, C., Dufour, S., 2009 Dopamine inhibition of eel reproduction, in: van den Thillart, G., Dufour, S., Rankin, J.C. (Eds.), Spawning migration of the European eel. Springer, the Netherlands, pp. 279-307.
Zhou, H., Tong, Z., McLeod, J.F., 2004. "Cocktail" approaches and strategies in drug development: Valuable tool or flawed science? J. Clin. Pharmacol. 44, 120-134.

Tables

Table 1. Experimental conditions during the simulated reproductive migration of European eel in freshwater (FW) and seawater (SW).

	Sampling group	Sampling after start experiment (days)	Temperature (°C)	Light (L:D)	Salinity (‰)	Water velocity (m s^{-1})
prior to experiment	START	0	23.5	24:0	0	0
control for M1	C1	14	23.5	24:0	0	0
FW migration	M1	14	11.7	8:16	0	0.57
control for M2	C2	77	11.5	8:16	0	0
SW migration	M2	77	10.1 – 11.7*	0:24	29.2	0.57

*10.1 °C for 8 hrs day^{-1} and 11.7 °C for 16 hrs day^{-1}.

Table 2. Male European eel parameters (average ± se) during a simulated reproductive freshwater and seawater migration.

Parameters	(START) *n*=10		control (C1) *n*=10		FW migration (M1) *n*=10		control (C2) *n*=10		SW migration (M2) *n*=10	
External										
BL (cm)	42.7	± 0.9	43.4	± 0.9	44.1	± 0.8	44.5	± 0.7	42.9	± 0.8
BW (g)	162	± 12	163	± 13	180	± 10	160	± 8	160	± 8
K	0.21	± 0.01	0.20	± 0.01	0.21	± 0.01	**0.18**	**± 0.01**	0.20	± 0.01
BGI	20.3	± 0.4	19.9	± 0.4	20.4	± 0.4	**18.7**	**± 0.4**	19.9	± 0.3
PFLI	44.8	± 1.2	45.3	± 0.9	44.7	± 0.9	44.2	± 1.4	44.9	± 2.6
EI	11.4	± 0.7	11.9	± 0.6	12.0	± 0.6	12.3	± 0.4	**14.0**	**± 0.6**
Internal										
Hct (%)	35.4	± 2.3	38.8	± 0.6	**30.2**	**± 0.9**	30.0	± 1.2	32.0	± 1.1
GW (g)	0.041	± 0.014	**0.088**	**± 0.017**	0.134	± 0.019	**0.058**	**± 0.006**	**0.084**	**± 0.007**
GSI	0.026	± 0.009	**0.053**	**± 0.009**	0.076	± 0.010	**0.036**	**± 0.002**	**0.052**	**± 0.003**
HSI	0.97	± 0.04	**0.86**	**± 0.04**	**1.10**	**± 0.06**	1.02	± 0.06	1.07	± 0.07
T (pg ml^{-1})	9.3	± 3.6	8.2	± 2.1	**38.9**	**± 7.5**	26.7	± 5.7	**57.5**	**± 4.3**
LH (CPS)	441	± 41	**667**	**± 89**	667	± 61	**421**	**± 36**	678	± 232
FSH (CPS)	96	± 5	116	± 14	114	± 11	102	± 6	114	± 8

Parameters were statistically tested pair-wise against the parameters directly left, e.g. parameters from C1 were compared with parameters from START, M1 was compared with C1 etc. Significant (p<0.05) differences are indicated in bold. Parameters shown are body length (BL); body weight (BW); Fulton's condition factor (K); body girth index (BGI); pectoral fin length index (PFLI); eye index (EI); haematocrit (Hct); gonad weight (GW); gonadosomatic index (GSI); hepatosomatic index (HSI); plasma testosterone (T); plasma luteinizing hormone (LH); and plasma follicle-stimulating hormone (FSH). Both LH and FSH are shown in fluorescence counts per second (CPS; see sections 2.3 and 3.1).

Table 3. Female European eel parameters (average ± se) during a simulated reproductive freshwater and seawater migration.

Parameters	(START)		control (C1)		FW migration (M1)		control (C2)		SW migration (M2)	
	n=10		*n*=10		*n*=10		*n*=10		*n*=10	
External										
BL (cm)	70.1	± 1.8	72.0	± 2.1	71.9	± 1.5	68.9	± 2.6	72.9	± 2.0
BW (g)	751	± 61	805	± 92	784	± 54	688	± 79	848	± 75
K	0.21	± 0.01	0.21	± 0.01	0.21	± 0.01	0.20	± 0.00	0.21	± 0.01
BGI	20.5	± 0.4	19.8	± 0.6	20.1	± 0.3	19.7	± 0.3	20.2	± 0.5
PFLI	39.2	± 1.3	39.1	± 0.8	37.3	± 1.8	39.9	± 1.2	40.7	± 1.3
EI	9.7	± 0.5	10.3	± 0.4	10.1	± 0.3	11.4	± 0.7	11.5	± 0.6
Internal										
Hct (%)	38.5	± 1.5	38.1	± 1.1	**34.1**	**± 1.3**	36.1	± 1.6	33.3	± 2.1
GW (g)	5.5	± 0.8	7.0	± 1.3	7.9	± 0.7	7.3	± 1.2	**11.8**	**± 1.1**
GSI	0.70	± 0.07	0.81	± 0.09	1.01	± 0.04	1.00	± 0.10	**1.40**	**± 0.06**
HSI	0.93	± 0.06	0.88	± 0.05	**1.10**	**± 0.04**	1.04	± 0.04	1.08	± 0.05
T (pg ml^{-1})	18.3	± 5.7	15.1	± 4.9	**37.7**	**± 7.2**	26.4	± 4.7	28.9	± 7.8
LH (CPS)	438	± 52	**1217**	**± 318**	609	± 32	577	± 62	693	± 258
FSH (CPS)	114	± 12	162	± 49	115	± 5	112	± 5	137	± 19

For statistics and abbreviations see Table 2.

Figure 1. The swim-flume. Treated eels were housed in the swimming compartment (compartment where the water current arrives through the outer curve). During the experiment, the flume was covered with thick black foil to shield the eels from exogenous light and artificial light was only provided during FW-migration.

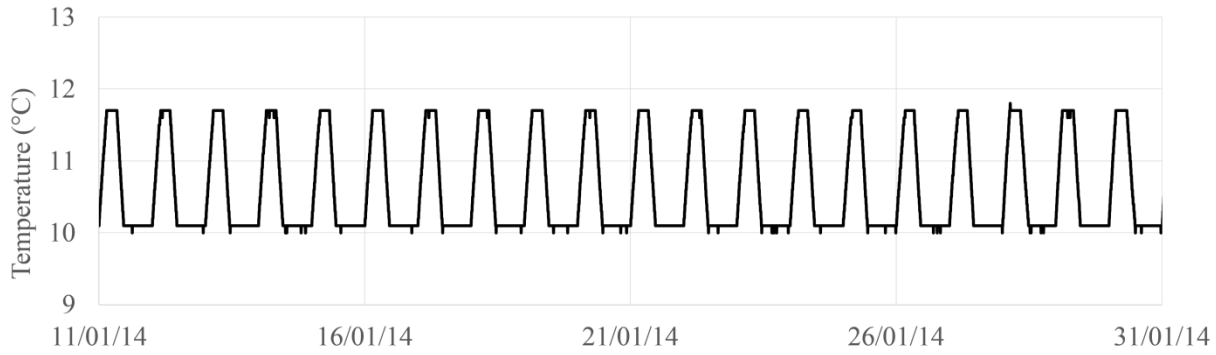

Figure 2. The daily temperature profile in the swimming flume during simulated seawater migration. Temperature was fluctuated between 11.7 °C for 8 h day^{-1} (0:00 h to 8.00 h) and 10.1 °C for 16 h day^{-1} (8:00 h to 0:00 h) to simulate the average temperature profile that eels experience during the daily vertical migrations in the ocean. Note that data of only 20 days of the experimental period are shown.

Ib. Gesimuleerde migratie van gefeminiseerde alen

The effects of a simulated migration under a natural photothermal protocol on the sexual maturation of feminised European eels *(Anguilla anguilla)*

1. Introduction

A. anguilla (Linnaeus, 1758) undertakes a 6,000 km reproductive migration from fresh water habitats in Europe and North-Africa to the presumed spawning areas in the Sargasso Sea (Schmidt, 1923; Tesch, 2003). From this area, newly hatched larvae develop into *Leptocephali* (e.g. feeding larvae) which drift by the current (NE component of the North Atlantic Gyre) towards the European coasts and other parts of their distribution areas (Lecomte-Finiger, 1994; Arai *et al.,* 2000). When they reach the continental shelf, they metamorphose into glasseels that enter brackish and fresh water systems begin their continental phase of life (Tesch, 2003). During this phase, eels grow as elvers and then they become yellow eels, the preponderant stage of their life cycle that can last from 3 (males) or 6 (females) up to 50 years during which lipid reserves are built up (Tesch, 2003; Kirkegaard *et al.,*2010).

Pre-pubertal yellow eels in rivers undergo morphological and physiological modifications that anticipate the seawater spawning migration during a reversible process known as "silvering". These modifications include external ones such as change in skin colour including darkening of the dorsal side and silvering of the belly and enlargement of the eyes, and internal ones such as degeneration of the gastrointestinal tract, higher branchial chloride cell density and gonadal development (reviewed by Durif *et al.,* 2009). At the onset of their reproductive migration, sexual maturation appears to be dually blocked by a strong dopaminergic inhibition in the brain (DA; Vidal *et al.,* 2004; Weltzien *et al.,* 2009; reviewed by Dufour *et al.,* 2003) and through a deficiency in gonadotropin realising hormone (GnRH; Weltzien *et al.,* 2009). These blockages interrupt steroidogenesis and gametogenesis in the gonads due to lack in release of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Weltzien *et al.,* 2009). However, the inhibition at the pituitary level can be artificially circumvented through hormonal treatments with gonadotropins that stimulate gonadal development and thereby permit to produce eggs and offspring (Fontaine *et al.,* 1964). In a similar species, *Anguilla japonica* (Temminick and Schlegel, 1846), scientists successfully completed the lifecycle in captivity (Ohta *et al.,* 1997; Kagawa *et al.,*2005) applying hormonal injections, stripping of the gametes, artificial fertilization of eggs and rearing and exogenous feeding of the larvae. Regarding the European eel, recent experimental studies resulted in production of offspring living up to 20/22 days (Tomkiewicz *et al.,* 2014, pers. comm.). Currently, full artificial maturation in male eels is induced by injection of human chorionic gonadotropin (Fontaine *et al.,* 1936). Inducing complete artificial maturation of females requires weekly injections with carp pituitary extract (CPE) or salmon pituitary extract (SPE) over a period of 3 to 6 months (Fontaine *et al.,* 1964; Boëtius and Boëtius, 1980; Pedersen, 2003, 2004; Palstra *et al.,* 2005). These procedures cause developmental abnormalities during the maturation process due to the weekly handling stress and unnaturally high peaking levels of hormones which are injected in eels that not even in the pubertal phase. Furthermore, CPE contains other hormones than gonadotropins that may affect sexual maturation. Because of that, there is a strong need to replace and/or reduce the artificial trajectory of hormonal injections by a natural trajectory of natural triggers.

Recently, more insight has been gained into the effects of swimming exercise and environmental conditions as experienced during migrations such as photoperiod, salinity, temperature and pressure. During freshwater migration, swimming exercise in European eel promotes lipids deposition during early oocyte development but, swimming in sea water suppresses any further maturation (Palstra *et al.,* 2007, 2009; Palstra and Van den Thillart, 2010). Exercise also acts as an inhibitor for the ovarian development and as down-regulator of the ovarian transcriptome in *O.mykiss* (Palstra *et al.,* 2010). In contrast to female eels, swimming exercise in males stimulates spermatogenesis by up-regulating GnRH levels resulting in LHß production of the pituitary (Palstra *et al.,* 2008a). Maturation in eels can be affected by factors such as salinity (Nilsson *et al.,* 1981; Kagawa *et al.,* 1998; Kagawa, 2003), light (Nilsson *et al.* 1981, Mordenti*et al.,*2014) and temperature (Böetius and Böetius, 1967 ; Perez 2011). Aarestrup *et al.*

(2009) reported on the diel vertical migrations that eels perform which will expose the eels to diel alternations in water temperature.

In some reproductive experiments to breed *A. anguilla*, wild eels were used as broodstock, because of their optimal gamete quality (Pedersen, 2004; Palstra *et al.,* 2005) but this approach is not sustainable and several disease problems using these eels were encountered (Pedersen, 2004). Tachiki *et al.*, (1997) established a method that permits to obtain feminisation by applying 17-β Estradiol(E2) coated feed. Feminisation accelerates oocyte development and increases their competences to respond to pituitary extract as compared to non-feminised cultured eels (Ijiri *et al.,* 1998). Feminised eels grow faster and respond better to hormonal injections, ready to use already after 14 month since the glass eel stage (Yamada *et al.,* 2006; Chai *et al.,* 2010). In E2 treated *A. japonica*, the number of weekly injections required to reach the final maturation was lower than in normally cultivated eels (Respectively 16.6 *vs*. 18.6 injections) but still higher as in wild caught females (10.0 injections). Consequently, the E2 feminisation protocol seems to be a promising tool in order to improve European eel broodstock maturation and to reduce the number of invasive treatments which injections are.

The use of cultured eels as broodstock has other disadvantages. Tomkiewicz *et al., (*2012) and Støttrup *et al.,*(2013) reported differences in total content and ARA/EPA (respectively arachidonic and eicosapentanoic acid) ratios between farmed and wild eels. In particular, eggs of farmed eels showed low percentage of ARA and higher EPA in contrast to the eggs of wild eels. Eel ovaries and eggs are characterized by high levels of ARA that are selectively incorporated in *A. japonica* ovaries evidencing the importance of this PUFA (Poly Unsaturated Fatty Acid) in the reproductive process of Anguillids and other Teleostei (Fernandez-Palacios *et al.,* 2011). ARA uptake by the gonadal polar lipids observed in *A. japonica* and *A. anguilla* confirms the essential role that this fatty acid plays in eel maturation (Furuita *et al.,* 2007; Støttrup *et al.,* 2013). ARA derived prostaglandines and eicosanoids play important roles in ovulation acting as agonists of GnRH (Mustafa and Srivastava, 1989).

Previous studies have tested the effects of single factors such as temperature, light, salinity, pressure and exercise on the sexual maturation of eels (Van den Thillart *et al.,* 2009; Tomkiewicz, 2012; Butts *et al.,* 2014, Mordenti *et al.,* 2012; Parmeggiani *et al.,* 2014). In this study, we have assessed the multifactorial effects of a simulated migration under mimicked photothermal conditions on the sexual maturation in feminised European eels. Specifically, migration was simulated trough subjecting eel broodstock to forced sustained swimming at optimal speed (U_{opt}) under natural phototermal conditions, first in fresh water (FW) for two weeks and then in salt water (SW) for 6 weeks. Before the simulated migrations, fish were fed with a wild fish diet and monitored monthly for changes in size and silvering. These changes and changes in fat levels were determined in eels before and after FW migration, and after SW migration when hormonal injections were started to bring eels to reproduction. We hypothesize that a simulated migration under a natural photothermal protocol will stimulate the early sexual maturation of feminised European eels, even more pronounced as in old (4-5 year old) cultured female eels that have been subjected to a similar protocol (Mes *et al.,* 2016).

2. Materials and Methods

Ethics

Applied procedures were in agreement with the current laws of Netherlands and were approved by the Animal Experimental Committee (DEC) of the Wageningen University and Research Centre situated in Lelystad (The Netherlands) under number 2014216.

Experimental set-up

Migration was simulated in the 3.600 L Brett-type swim flume as described before (Palstra *et al.,* 2015). Briefly, one of the straights of the oval-shaped flume was divided in two equal and parallel 525 L compartments (200 X 35 X 70 cm each) in order to swim the eels. Water flow was created by an impeller positioned in the straight opposite to the compartments. The impeller was driven by a propeller that moved a constant water flow through the outer compartment where the fish were swum. Every hour, the whole water volume of the system was led trough a drum filter, trickle filter, 200 L moving bed biofilm reactor (MBBR), a protein skimmer and UV-filter. Water renewal rate in the flume was 60 L h^{-1}.

Experimental fish and conditions

Feminised eels (*N*= 18) were obtained from Leiden University where they have been raised from the glass eel stage in fresh water under a 24L:0D photoperiod. At the IMARES facilities, experimental fish were placed in a RAS Unit 250 L tank set at a water temperature of 25 C° and at a 24L:0D photoperiod. In this tank, water was continuously led over a 75 L MBBR into a 100L sump. Water from the sump was run over a heat exchanger (HC-1000A, Hailea, Guangdong, China, adapted by Climate4u.nl, Valkenswaard, the Netherlands) and UV-filter (AquaCristal UV-C 5W, JBL GmbH KG, Neuhofen, Germany), before being pumped back into the fish tank. Water renewal was 20 L h^{-1}. Under these conditions, eels were kept for a period of five months (from January 23rd to June 12th) and fed with mackerel fillet one time per day. During the entire feeding phase, eels have eaten 8.360 kg of food that is 411 g per individual. Fresh mackerels were monthly purchased from a local fish shop (Vishandel van As Zeeland B.V., Yerseke), filleted, chopped and daily feeding portions were kept frozen at -20 °C. During the entire feeding phase eels were monitored daily for well-being. Water quality parameters were measured weekly. O_2 level was 8.35 ± 0.11 mg L^{-1} (between 8.07 and 8.61 mg L^{-1}), pH was 8.29 ± 0.01 and salinity was 0 PSU. N-NH_4 averaged 0 ± 0.00 mg L^{-1} whereas N-NO_2 was 0.01 ± 0.00 mg L^{-1} (0 - 0.025 mg L^{-1}). During this period of five months, every 30 days eels were monitored for silvering and biometrical measurements were taken at this time. On June 12th, eels were transferred from the tank to the swim flume. Together with a second batch of feminized eels (N=29) and male cultured eels (N=100), eels were housed in the swimming compartment. After 24 hours of acclimatization, motor frequency was gradually increased to drive the propeller creating the desired water flow speed of 57 cm s^{-1} (the optimal swimming speed for male eels; Burgerhout *et al.,* 2014) which corresponded to 0.89 BL s^{-1} for the eighteen experimental eels. Flume conditions during fresh water migration were set at 11.7 °C, a salinity of 0 PSU and a light regime of 8L:16D in order to simulate the temperature and light availability during peak migratory activity in wild eels populations (Bruijs *et al.,* 2003; Bruijs and Durif, 2009). After 14 days of swimming in fresh water, eels had covered a distance of 689 Km. Subsequently, salinity in the flume was gradually increased up to 31.5 ± 0.07 PSU during three days adding natural, UV-treated seawater from the estuary Oosterschelde. In order to simulate the thermic profile as experienced by migrating eels during seawater migration, temperature in the flume was fluctuated in controlled fashion between 10.1 °C for 16 h day^{-1} (from 08:00 to 00:00) and 11.7 °C for 8 h day^{-1} (from 00:00 to 08:00) (Aarestrup *et al.,* 2009). Water in the flume took 2 h and ± 10 m to heat from 10.1 °C to 11.7 °C and 2h ± 3 m viceversa. Eels were swimming in darkness. After 63 days of simulated oceanic migration, eels had swam 3,201 km. Eels were then transferred back to a RAS 250 L circular tank in order to start hormonal treatments to achieve full maturation. In the swimming flume, water quality was monitored every five minutes for temperature, pH, O_2 and salinity. During fresh water migration (FW), temperature was 11.72 ± 0.02 °C, pH was 8.23 ± 0.02, O_2 was 10.71 ± 0.06 mg L^{-1} and salinity was 0 PSU. During seawater migration (SW), temperature was 11.71 ± 0.5 °C at night and 10.1 ± 0.0°C during the day, pH was 7.64 ± 0.0, O_2 was 9.52 ± 0.11 mg L^{-1} and salinity was 31.5 ± 0.07 PSU. During both migration phases water quality was monitored weekly for NH_4 and NO_2. N-NH_4averaged 0.01 ± 0.01 mg L^{-1} (0-0.4 mg L^{-1}) and N-$NO_2$was 0.11 ± 0.06 mg L^{-1} (0-0.30). Eels were not fed during the entire simulated migration experiment.

Biometrics

Per monitoring event, total body lenght (TL), body weight (BW), body girth (BG), horizontal and vertical eye diameter (respectively Edh and Edv) and pectoral fin lenght (PFL) were measured. Fat percentage was determined only during the last three sampling points (START, FW and SW) using a fish fatmeter (Distell.com FFM-992, city). The following indices were calculated: Fulton's condition factor (Fulton, 1911), Body Girth Index (BGI; Palstra and van den Thillart, 2009), Eye Index (EI; Pankhurst, 1982) and Pectoral Fin Lenght Index (PFLI; Durif *et al.,* 2003).

Fulton's condition factor **(K) : 100 × BW TL^{-3}**
Where BW is the body weight (g) and TL is the total lenght (cm).

Body girth index **(BGI): 100 × BG BW^{-1}**
Where BW is the body weight (g) and BG is the body girth (cm).

Eye index **(EI): 100 × (((EDh + EDv) × 0,25)^2 π × (10 × BL)$^{-1}$)**
Where EDh is the horizontal diameter of the eye, EDv is the vertical diameter of the eye and BL is the body lenght.

Pectoral fin lenght index **(PFLI): 100 × PFL BW^{-1}**
Where BW is the body weight (g) and PFL is pectoral fin lenght (cm).

Statistics
All data analyzed were normally distributed (Shapiro-Wilk tests). In order to test any significant progression in sexual maturation process during the entire experimental period, all morphometric indices (K, BGI, EI and PFLI) were pair-wise compared using paired one-tailed Student's t-test comparing each value with the subsequent value in the time series (e.g. April *vs.* March, May *vs.* April etc). Average fat percentages were pair-wise compared using paired one-tailed Student's t-test for the three sampling points during the simulated migration only (START *vs.* FW and FW *vs.* SW). All data are shown as average ± SE and statistical differences were considered significant at $P<0.05$

3. Results

Feeding phase
In the five months prior to the simulated migration, female eels were of similar size (63.7 ± 0.83 cm) and did not show any significant change in K nor BGI ($P> 0.05$). PFLI changed significantly only between February and March (from 41.20 ± 0.94 to 42.85 ± 0.90; $P=0.0236$; Fig. 1). BWI increased during the feeding phase but significantly only between April and May ($P=0.03$). EI increased significantly from 10.40 ± 0.27 in February to 11.80 ± 0.27 in March ($P<0.001$) but decreased significantly again from 11.65 ± 0.28 in April to 11.34 ± 0.25 in May ($P= 0.0035$). At the start point of the FW migration eels had an eye index of 10.94 ± 0.25.

Swimming behaviour
During the whole simulated migration (80 days) eels aggregated in a school on the bottom of the swimming flume and displayed group-wise positive rheotactic swimming. Total distance covered by the eels during this period was 3,940 Km. No evident signs of stress, disease or infections were observed during the simulated migration. Mortality was not observed for the eighteen experimental eels but occurred among the other eels (*N*= 1 females, *N*= 2 males) which involved smaller eels that were not willing or able to swim.

Freshwater migration
After two weeks of simulated migration, feminized FW migrants showed lower K values (0.21 ± 0.00 *vs.* 0.22 ± 0.00; $P= 0.0127$), fat percentages (23.86 ± 0.25 % *vs.* 24.60 ± 0.39 %; $P= 0.0039$) and BGI (0.21 ± 0.00 *vs.* 0.22 ± 0.00 ; $P= 0.0347$) as compared to the eels from the START group. No differences in PFLI (43.34 ± 0.73 *vs.* 43.46 ± 0.74; $P= 0.8324$) were observed during the fresh water migration. Neither did EI change significantly during fresh water migration (11.00 ± 0.25 *vs*. 10.94 ± 0.25; $P= 0.5284$).

Seawater migration
After seawater migration (65 days), K decreased significantly as compared to the status at the start of SW migration (0.21 ± 0.00 *vs.* 0.20 ± 0.00; $P< 0.0001$). The BGI was lower after the seawater migration than before (0.20 ± 0.00 vs 0.21 ± 0.00; $P< 0.0001$). Fat percentage decreased from 23.86 ± 0.25 % to 20.58 ± 0.34 ($P<0.0001$) after the simulated oceanic migration and in this period EI increased highly significantly from 11.00 ± 0.25 to 13.41 ± 0.31 ($P< 0.0001$). PFLI was also significantly higher after SW migration than before (45.70 ± 0.77 *vs.* 43.46 ± 0.74; $P= 0.0025$).

4. Discussion and Conclusions

The environmental factors that European eels experience during their migration from freshwater feeding habitats towards their spawning area in the Sargasso Sea may act as cues for the progression of sexual maturation.
These cues, such as temperature (Perez *et al.,* 2011), light regime (Nilsson *et al.* 1981), salinity (Kagawa *et al.,* 1998; Kagawa 2003), pressure (Sébert *et al.,* 2007) and exercise (Palstra *et al.,* 2007, 2008, 2010) have been shown to influence the sexual maturation of female eels. Recently, a study conducted by Mes *et al.,* (2016) investigated for the first time the combined effects of these factors (except for

pressure) during a simulated migration of 3,792 Km. In that study, both farmed male and females eels, showed enhancement of silvering and maturation during simulated freshwater and seawater migrations. Similarly, in this study, we have assessed the multifactorial effects of a simulated migration of 3,940 km on the sexual maturation in feminised European eels. Feminised European eels showed a similar enhanced progression in silvering but also appeared to respond better than the eels in the previous experiment

Simulated freshwater migration

No growth was observed during the feeding period. At the start of this period, experimental eels were already considered as silver on basis of the EI (Pankhurst, 1982). Wild European silver eels leave their FW habitats probably driven by the decrease in hours of light and water temperature as appearing during autumn (reviewed by Bruijs and Durif., 2009). This group-wise migration involves both sexes which is of importance as it has been shown that sexually mature eels promote gonadal development in neighbouring males (Huertas *et al.,* 2006) probably by pheromone-mediated mechanisms. For this reason, 100 males swam together with the experimental females under mimicked photothermal conditions, e.g. at a photoperiod of 8L:16D and temperature of 11.7 °C. The applied temperature is within the thermal range experienced by eels at the start of their downstream migration in Norway, Spain, France and Netherlands (reviewed by Bruijs and Durif, 2009). In eels, energetic reserves obtained prior to the spawning migration are pivotal. *A. anguilla* spends an average amount of 67% of the initial lipid stores on reproduction (although individual variation is extensive; Palstra & van den Thillart, 2010). In female eels, 39 % of the initial body fat reserve is used for migration whereas 28% is used for development of eggs (Palstra and van den Thillart, 2010). Fat percentages between 25-35% are characteristic of silver eels (e.g. Larsson *et al.,* 1990; Kamstra and Van Heeswijk, 1996; Clevestam *et al.,* 2011) and our results are consistent with this because after FW migration fat levels decreased from 24.60 ± 0.39 % to 23.86 ± 0.25 %.

FW swimming has been shown to enhance silvering in females with associated enlargement of the eyes and lipid deposition in the oocytes (reviewed by Palstra *et al.,* 2009; Palstra and van den Thillart, 2010; Palstra *et al.,* 2014). During the two weeks of simulated FW migration in this study, EI did not change. Mes *et al.,* (2016), using the same photothermal protocol in 4 to 5 years old cultured eels, also did not find any significant advance in maturation of females after simulated FW migration.

Simulated seawater migration

Since no European eels have ever been caught at late stages of the SW migration, few information is available on the effects of SW migration on maturation in nature. Recently, Kurogi *et al.,* (2011) reported the first captured post-spawning *A. japonica* at the Southern Western Mariana Ridge. During SW migration in our experiment, a daily fluctuating temperature profile was applied because female eels migrate vertically between 200 metres depth and 11.7 °C at night and 700 metres depth and 10.2 °C during the day during oceanic migration (Aarestrup *et al.,* 2009). Mikawa *et al.* have shown in *A. japonica* that fluctuations in temperature between 5 and 15 °C induced ovarian development (GSI= 8) but these results were on based on a single eel and could not be repeated (Palstra, pers. comment).

Differently to the FW one, a simulated SW migration, did stimulate sexual maturation influencing significantly all the analyzed parameters. This is in agreement with a previous study conducted using 4-5 years old cultured females and the same experimental protocol (Mes *et al.,* 2016) that showed increased EI and GSI after SW migration. We can also state, as hypothesized, that feminised eels responded better to the same experimental protocol than the cultured ones since in that study, Mes et al. (2016) obtained EI values of 11.50 and PFLI values of 40.70. In this study, these values were higher at an average of 13.41 that is 16.61 % higher and 45.70 for PFLI that is 12.29 % higher than the ones obtained by Mes *et al.* 2016. So, the 17-B estradiol feminisation protocol seems to be a promising tool in order to improve European eel broodstock conditioning.

5. References

Acou, A., Boury, P., Laffaille, P., Crivelli, A., Feunteun, E., 2005. Towards a standardized characterization of the potentially migrating silver European eel

(Anguilla anguillaL.). ArchivfürHydrobiologie 164, 237–255.

Aoyama, J., Miller, M.J., 2003. The Silver Eel. In: Aida, K., Tsukamoto, K., Yamauchi, K. (Eds.), Eel Biology. Springer-Verlag, Tokyo, pp. 107–117.

Antonucci, Francesca, Corrado Costa, Jacopo Aguzzi, and Stefano Cataudella. "Ecomorphology of morpho-functional relationships in the family of Sparidae: a quantitative statistic approach." *Journal of Morphology* 270, no. 7 (2009): 843-855.

Aoyama, J., Miller, M.J., 2003. The Silver Eel. In: Aida, K., Tsukamoto, K., Yamauchi, K. (Eds.), Eel Biology. Springer-Verlag, Tokyo, pp. 107–117.

ARAI, T.; OTAKE, T.; TSUKAMOTO, K. Timing of metamorphosis and larval segregation of the Atlantic eels Anguilla rostrata and A. anguilla, as revealed by otolith microstructure and microchemistry. *Marine Biology*, 2000, 137.1: 39-45.

BAEZA, R., et al. Effect of thermal regime on fatty acid dynamics in male European eels (Anguilla anguilla) during hormonally-induced spermatogenesis. *Aquaculture*, 2014, 430: 86-97.

BOËTIUS, Inge; BOËTIUS, Jan. *Studies in the European eel, Anguilla anguilla [L.]: Experimantal induction of the male sexual cycle, its relation to temperature and other factors*. Andr. Fred. Høst & Søn, 1967.

BOESGAARD, Lars; NIELSEN, Michael E.; ROSENKILDE, Per. Moderate exercise decreases plasma cortisol levels in Atlantic salmon (Salmosalar). *Comparative Biochemistry and Physiology Part A: Physiology*, 1993, 106.4: 641-643.

BROWN, Elliot J., et al. Do swimming fish always grow fast? Investigating the magnitude and physiological basis of exercise-induced growth in juvenile New Zealand yellowtail kingfish, Seriolalalandi. *Fish physiology and biochemistry*, 2011, 37.2: 327-336.

BRUIJS, Maarten CM; DURIF, Caroline MF. Silver eel migration and behaviour. In: *Spawning Migration of the European Eel*. Springer Netherlands, 2009. p. 65-95.

BURGERHOUT, Erik, et al. First artificial hybrid of the eel species Anguilla australis and Anguilla anguilla. *BMC developmental biology*, 2011, 11.1: 16.

BUTTS, Ian AE, et al. Standardization of fertilization protocols for the European eel, Anguilla anguilla. *Aquaculture*, 2014, 426: 9-13.

BUTTS, Ian Anthony Ernest, et al. Impact of dietary fatty acids on muscle composition, liver lipids, milt composition and sperm performance in European eel. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 2015, 183: 87-96.

CHAI, Yu, et al. The relationship between the developmental stage of oocytes in various seasons and the quality of the egg obtained by artificial maturation in the feminized Japanese eel Anguilla japonica. *水産増殖= Aquaculture Science*, 2010, 58.2: 269-278.

CHOW, Seinen, et al. Japanese eel Anguilla japonica do not assimilate nutrition during the oceanic spawning migration: evidence from stable isotope analysis. *Mar EcolProgSer*, 2010, 402: 233-238.

DAVISON, W.; HERBERT, N. A. Swimming-enhanced growth. In: *Swimming physiology of fish*. Springer Berlin Heidelberg, 2013. p. 177-202.

DAVISON, William. The effects of exercise training on teleost fish, a review of recent literature. *Comparative Biochemistry and Physiology Part A: Physiology*, 1997, 117.1: 67-75.

DAVISON, William. The effects of exercise training on teleost fish, a review of recent literature. *Comparative Biochemistry and Physiology Part A: Physiology*, 1997, 117.1: 67-75.

DAVISON, W.; HERBERT, N. A. Swimming-enhanced growth. In: *Swimming physiology of fish*. Springer Berlin Heidelberg, 2013. p. 177-202.

Durif, C.M., Dufour, S., Elie, P., 2005. The silvering process of the eel: a new classification from the yellow resident stage to the silver migrating stage. J. FishBiol. 66, 1–19.

Durif, C.M., Dufour, S., Elie, P., 2006. Impact of silvering stage, age, body size and condition on the reproductive potential of the European eel. Mar. Ecol. Prog. Ser.607 327, 171–181.

DURIF, Caroline MF, et al. Seasonal evolution and individual differences in silvering eels from different locations. In: Spawning migration of the European eel. Springer Netherlands, 2009. p. 13-38.

FARRELL, A. P., et al. Effects of exercise training and coronary ablation on swimming performance, heart size, and cardiac enzymes in rainbow trout, Oncorhynchusmykiss. *Canadian journal of zoology*, 1990, 68.6: 1174-1179.

FERNÁNDEZ-PALACIOS, Hipólito, et al. Effects of broodstock diet on eggs and larvae. *Larval Fish Nutrition*, 2011, 151-181.

Fontaine, M., Bertrand, E., Lopez, E., Callamand, O., 1964. Sur la maturation des organs génitaux de l'anguillefemelle (Anguilla anguillaL.) et l'émissionspontanée des oeufsen aquarium. C. R. Hebd. Seanc. Acad. Sci. 259, 2907–2910.

FORSTER, Ian Paul; OGATA, Hiroshi. Growth and Whole-body Lipid Content of Juvenile Red Sea Bream Reared under Different Conditions of Exercise Training and Dietary Lipid. *Fisheries science*, 1996, 62.3: 404-409.

FURUITA, H., et al. Effect of n-3 and n-6 fatty acids in broodstock diet on reproduction and fatty acid composition of broodstock and eggs in the Japanese eel Anguilla japonica. *Aquaculture*, 2007, 267.1: 55-61.

HEINSBROEK, L. T. N., et al. A review on broodstock nutrition of marine pelagic spawners: the curious case of the freshwater eels (Anguilla spp.). *Aquaculture Nutrition*, 2013, 19.s1: 1-24.

IJIRI, Shigeho, et al. Pretreatment Reproductive Stage and Oocyte Development Induced by Salmon Pituitary Homogenate in the Japanese Eel Anguilla japonica. *Fisheries science*, 1998, 64.4: 531-537.

KAGAWA, Hirohiko, et al. The first success of glass eel production in the world: basic biology on fish reproduction advances new applied technology in aquaculture. *Fish Physiology and Biochemistry*, 2005, 31.2-3: 193-199.

KHAN, Javed R., et al. Accommodating the cost of growth and swimming in fish the applicability of exercise-induced growth to juvenile hapuku (*Polyprionoxygeneios*). *Frontiers in physiology*, 2014, 5.

KHAN, I. A.; LOPEZ, E.; LELOUP-HATEY, J. Induction of spermatogenesis and spermiation by a single injection of human chorionic gonadotropin in intact and hypophysectomized immature European eel (Anguilla anguilla L.). *General and comparative endocrinology*, 1987, 68.1: 91-103.

KIRKEGAARD, Eskild, et al. *European eel and aquaculture*. DTU Aqua. Institut for Akvatiske Ressourcer, 2010.

KNIGHTS, Brian. A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. *Science of the total Environment*, 2003, 310.1: 237-244.

JOBLING, Malcolm, et al. Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. Aquaculture international, 1993, 1.1: 20-46.

LECOMTE-FINIGER, Raymonde. The early life of the European eel. 1994.

LOKMAN, P.M.,Wass, R.T., Syter, H.C., Scott, S.G., Judge, K.F., Young, G., 2001. Changes in steroid hormone profiles and ovarian histology during salmon pituitary- induced vitellogenesis and ovulation in female New Zealand longfinned eels, Anguilla dieffenbachiiGray. J. Exp. Zool. 289, 119–129.

MES, D., et al. Forced sustained swimming exercise at optimal speed to enhance growth performance of yellotail kingfish (Seriolalalandi). In: *Book of abstracts of the International Congress on th Biology of Fish, 03-07 August 2014, Edinburgh, United Kingdom*. 2014. p. 162-162.

Mes, D., Dirks, R.P., Palstra, A.P. (2016) Simulated migration under mimicked photothermal conditions enhances sexual maturation of farmed European eel (Anguilla anguilla). Aquaculture 452: 367–372.

MILLIGAN, C. LOUISE; HOOKE, G. BRIAN; JOHNSON, CRYSTAL. Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *Journal of Experimental Biology*, 2000, 203.5: 921-926.

MORDENTI, Oliviero, et al. Controlled reproduction in the wild European eel (Anguilla anguilla): two populations compared. *Aquaculture international*, 2013, 21.5: 1045-1063.

MORDENTI, M., et al. Induction of Sexual Maturation in Wild Female European Eels (Anguilla anguilla) in Darkness and Light. 2012.

MUSTAFA, T.; SRIVASTAVA, K. C. Prostaglandins (eicosanoids) and their role in ectothermic organisms. In: *Advances in comparative and environmental physiology*. Springer Berlin Heidelberg, 1989. p. 157-207.

OHTA, Hiromi, et al. Artificial induction of maturation and fertilization in the Japanese eel, Anguilla japonica. *Fish Physiology and Biochemistry*, 1997, 17.1-6: 163-169.

PALSTRA, A. P., et al. Are dioxin-like contaminants responsible for the eel (Anguilla anguilla) drama?. *Naturwissenschaften*, 2006, 93.3: 145-148.

PALSTRA, A. P., et al. Swimming performance of silver eels is severely impaired by the swim-bladder parasite Anguillicolacrassus. *Journal of Experimental Marine Biology and Ecology*, 2007, 352.1: 244-256.

PALSTRA, Arjan; VAN GINNEKEN, Vincent; VAN DEN THILLART, Guido. Cost of transport and optimal swimming speed in farmed and wild European silver eels (Anguilla anguilla). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 2008, 151.1: 37-44.

PALSTRA, Arjan P., Denhi Schnabel, Maaike C. Nieveen, Herman P. Spaink, and Guido EEJM Van den Thillart. "Male silver eels mature by swimming." *BMC physiology* 8, no. 1 (2008): 14.

PALSTRA, Arjan P., et al. Saving energy to fuel exercise: swimming suppresses oocyte development and downregulates ovarian transcriptomic response of rainbow trout Oncorhynchusmykiss. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 2010, 299.2: R486-R499.

PALSTRA, Arjan P.; PLANAS, Josep V. Fish under exercise. *Fish physiology and biochemistry*, 2011, 37.2: 259-272.

VALENTE, Luísa MP, et al. What determines growth potential and juvenile quality of farmed fish species?. *Reviews in Aquaculture*, 2013, 5.s1: S168-S193.

PANKHURST, N.W., 1982. Relation of visual changes to the onset of sexual maturation in the European eel Anguilla anguillaL. J. Fish Biol. 21, 127–140.

PEREZ, L., Peñaranda, D.S., Dufour, S., Baloche, S., Palstra, A.P., Van Den Thillart, G.E., Asturiano, J.F., 2011. Influence of temperature regime on endocrine parameters and vitellogenesis during experimental maturation of European eel (Anguilla anguilla) females. Gen. Comp. Endocrinol. 174, 51–59.

PANKHURST, N.W., 1982. Relation of visual changes to the onset of sexual maturation in the European eel Anguilla anguillaL. J. Fish Biol. 21, 127–140.

PARMEGGIANI, Albamaria, et al. Effect of photoperiod on endocrine profiles in European eels (Anguilla anguilla) during artificially induced ovarian development. 2014.

PARMEGGIANI, A., et al. Effect of photoperiod on endocrine profiles and vitellogenin expression in European eels Anguilla anguilla during artificially induced ovarian development. *Theriogenology*, 2015, 83.4: 478-484.

PEDERSEN, Benedikte Hedegaard. Induced sexual maturation of the European eel Anguilla anguilla and fertilisation of the eggs. *Aquaculture*, 2003, 224.1: 323-338.

PEDERSEN, Benedikte Hedegaard. Fertilisation of eggs, rate of embryonic development and hatching following induced maturation of the European eel Anguilla anguilla. *Aquaculture*, 2004, 237.1: 461-473.

PEREZ, L., Peñaranda, D.S., Dufour, S., Baloche, S., Palstra, A.P., Van Den Thillart, G.E., Asturiano, J.F., 2011. Influence of temperature regime on endocrine parameters and vitellogenesis during experimental maturation of European eel (Anguilla anguilla) females. Gen. Comp. Endocrinol. 174, 51–59.

PERIAGO, Ma J., et al. Muscle cellularity and flesh quality of wild and farmed sea bass, Dicentrarchuslabrax L. *Aquaculture*, 2005, 249.1: 175-188.

SBAIHI, M., Fouchereau-Peron, M., Meunier, F., Elie, P., Mayer, I., Burzawa-Gerard, E.,Vidal, B., Dufour, S., 2001. Reproductive biology of the conger eel from the south coast of Brittany, France and comparison with the European eel. J. Fish Biol. 59, 302–318.

STØTTRUP, J. G., et al. Modification of essential fatty acid composition in broodstock of cultured European eel Anguilla anguilla L. *Aquaculture Nutrition*, 2013, 19.2: 172-185.

立木宏幸, et al. Effects of Oral Administration of Estradiol-17. BETA. to Young on Gonadal Sex and Growth of Japanese eel Anguilla Japonica. *水産増殖*, 1997, 45.1: 61-66.

TESCH, F.-W., 2003. The eel. Blackwell Publishing, Oxford.

TOMKIEWICZ, J. and Jarlbæk, H. 2008. Kunstig reproduktion af ål: Roe II ogIIb. DTU Aqua Report.

TOMKIEWICZ, Jonna, et al. *Reproduction of European Eel in Aquaculture (REEL): Consolidation and new production methods*. DTU Aqua. Institut for AkvatiskeRessourcer, 2012.

TUDORACHE, Christian; DE BOECK, Gudrun; CLAIREAUX, Guy. Forced and Preferred Swimming Speeds of Fish: A Methodological Approach. In: *Swimming Physiology of Fish*. Springer Berlin Heidelberg, 2013. p. 81-108.

TUDORACHE, Christian; DE BOECK, Gudrun; CLAIREAUX, Guy. Forced and Preferred Swimming Speeds of Fish: A Methodological Approach. In: *Swimming Physiology of Fish*. Springer Berlin Heidelberg, 2013. p. 81-108.

VAN GINNEKEN, Vincent JT; MAES, Gregory E. The European eel (Anguilla anguilla, Linnaeus), its lifecycle, evolution and reproduction: a literature review. *Reviews in Fish Biology and Fisheries*, 2005, 15.4: 367-398.

VAN GINNEKEN , V., Durif, C., Balm, S.P., Boot, R., Verstegen, M.W.A., Antonissen, E., van den Thillart, G., 2007. Silvering of European eel (Anguilla anguillaL.): seasonal changes of morphological and metabolic parameters. Anim. Biol. 57, 63–77.

VAN GINNEKEN , Vincent, Arjan Palstra, Pim Leonards, Maaike Nieveen, Hans van den Berg, Gert Flik, Tom Spanings, Patrick Niemantsverdriet, Guido van den Thillart, and AlbertinkaMurk. "PCBs and the energy cost of migration in the European eel (Anguilla anguilla L.)." *Aquatic Toxicology* 92, no. 4 (2009): 213-220.

VAN GINNEKEN, Vincent JT; VAN DEN THILLART, Guido EEJM. Physiology: Eel fat stores are enough to reach the Sargasso. *Nature*, 2000, 403.6766: 156-157.

VAN DEN THILLART, G. E. E. J., et al. Endurance swimming of European eel. *Journal of Fish Biology*, 2004, 65.2: 312-318.

VAN DEN THILLART, Guido; DUFOUR, Sylvie; RANKIN, J. Cliff. Spawning migration of the European eel. *Fish and Fisheries Series*, 2009, 30.

VIDAL, Bernadette, et al. Dopamine inhibits luteinizing hormone synthesis and release in the juvenile European eel: a neuroendocrine lock for the onset of puberty. *Biology of Reproduction*, 2004, 71.5: 1491-1500.

WELTZIEN, Finn-Arne, et al. Dopamine inhibition of eel reproduction. In: *Spawning Migration of the European Eel*. Springer Netherlands, 2009. p. 279-307.

WOODWARD, J. J.; SMITH, L. S. Exercise training and the stress response in rainbow trout, Salmogairdneri Richardson. *Journal of Fish Biology*, 1985, 26.4: 435-447.

YAMADA, S.; HORI, K.; SEOKA, M. Toward the improvements of broodfish management and egg quality of the Japanese eel, Anguilla japonica, for larviculture: Egg collection from young broodfish and effects of broodfish diet on egg fatty acid contents. *Bulletin of Fisheries Research Agency (Japan)*, 2006.

Figures

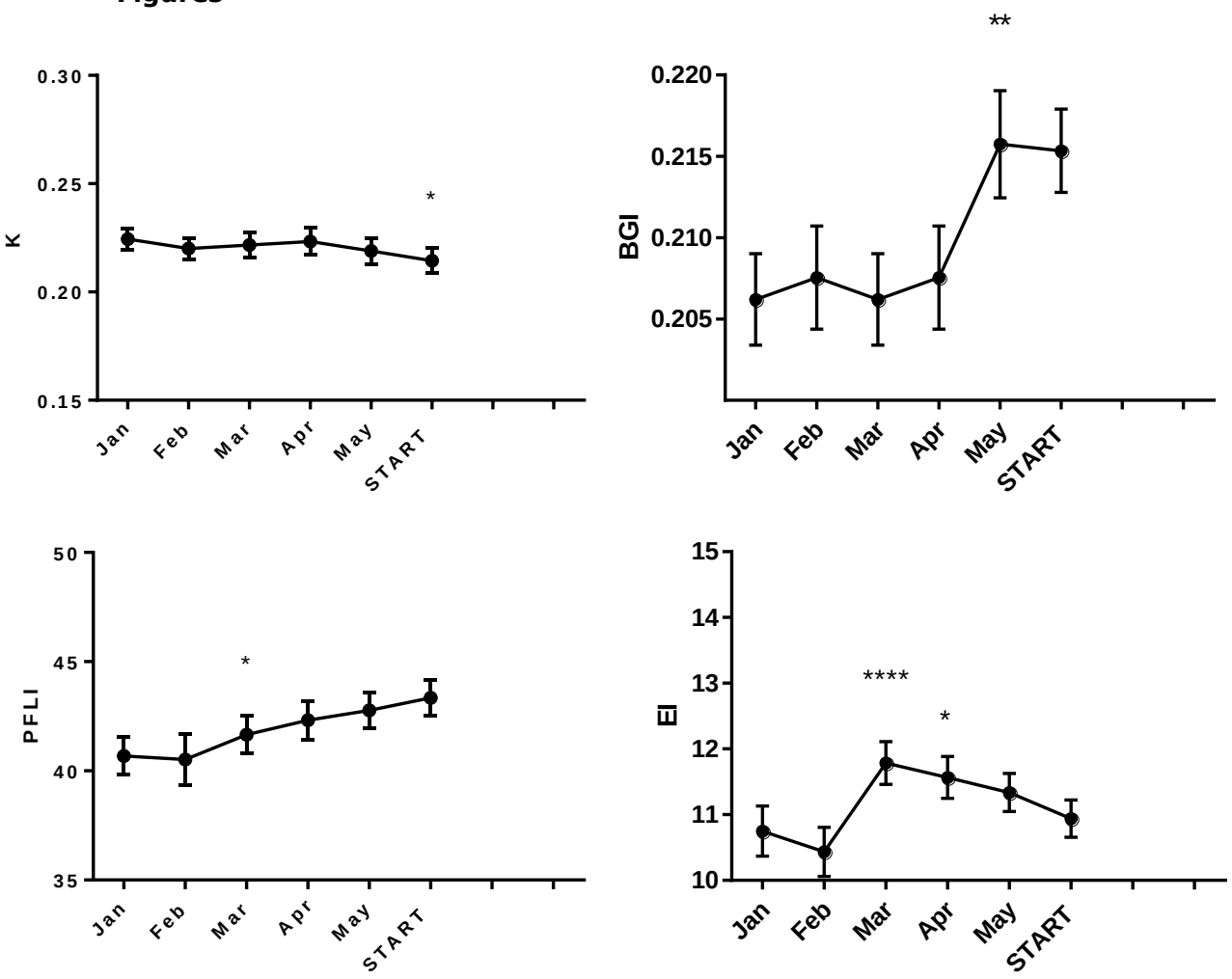

Figure 1. Fulton's condition factor (K), Body Girth Index (BGI), Pectoral Fin Lenght Index (PFLI) and Eye Index (EI) of 18 experimental eels during the feeding phase unitl the START of simulated migration. Results are obtained via paired, one tailed t-Student's Test and every month is compared with the previous one, errors bars represent SE. Asterisks bars indicate significant differences (* $p<0.05$; **$p<0.01$; ***$p<0.001$ and **** $p<0.0001$).

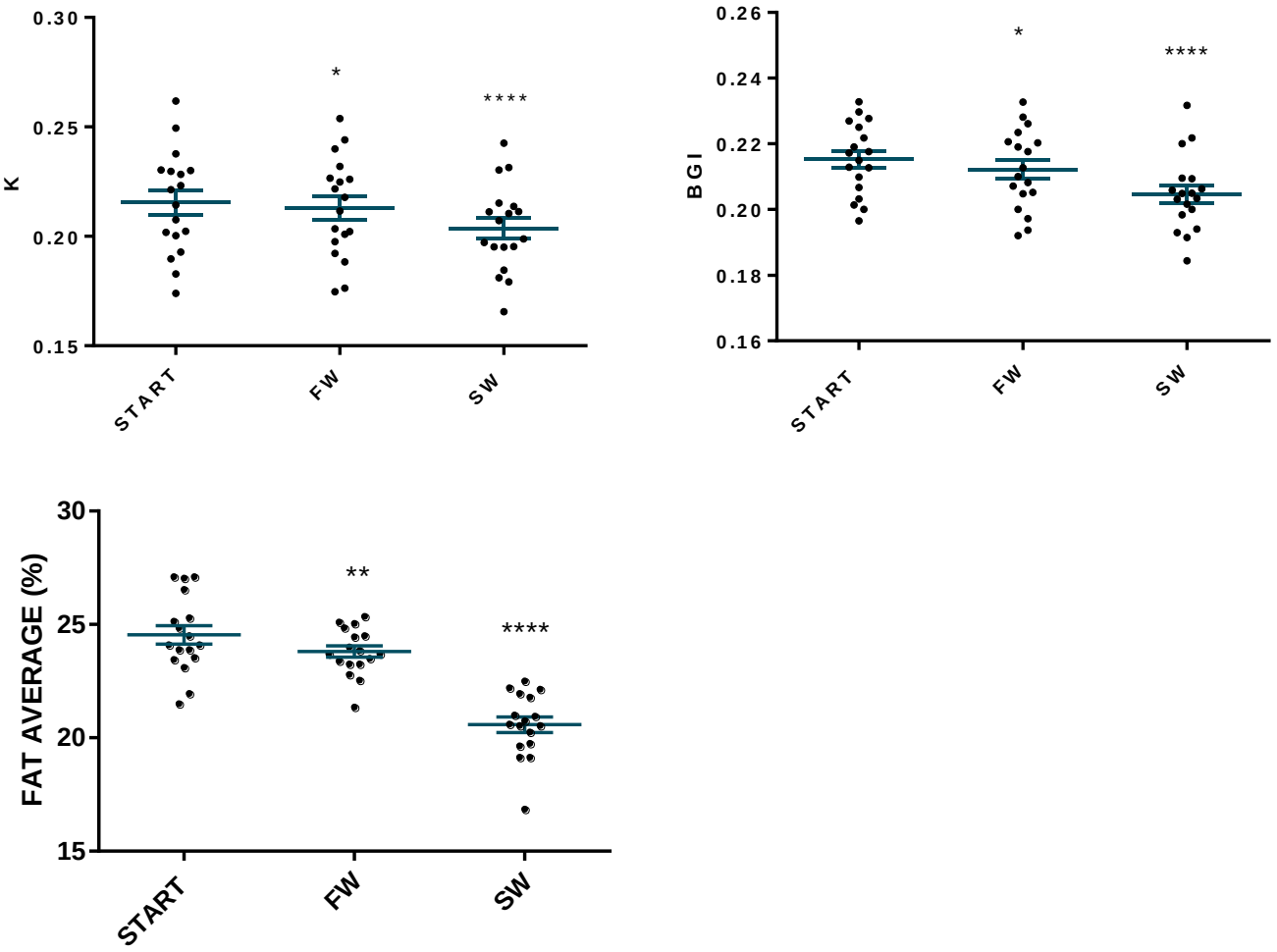

Figure 2. Fulton's condition factor (K), Body Girth Index (BGI) and fat percentage of 18 experimental eels duringsimulated migration. Results are obtained via paired, one tailed t-Student's Test and every month is compared with the previous one, errors bars represent SE. Asterisks bars indicate significant differences (* $p<0.05$; **$p<0.01$; ***$p<0.001$ and **** $p<0.0001$).

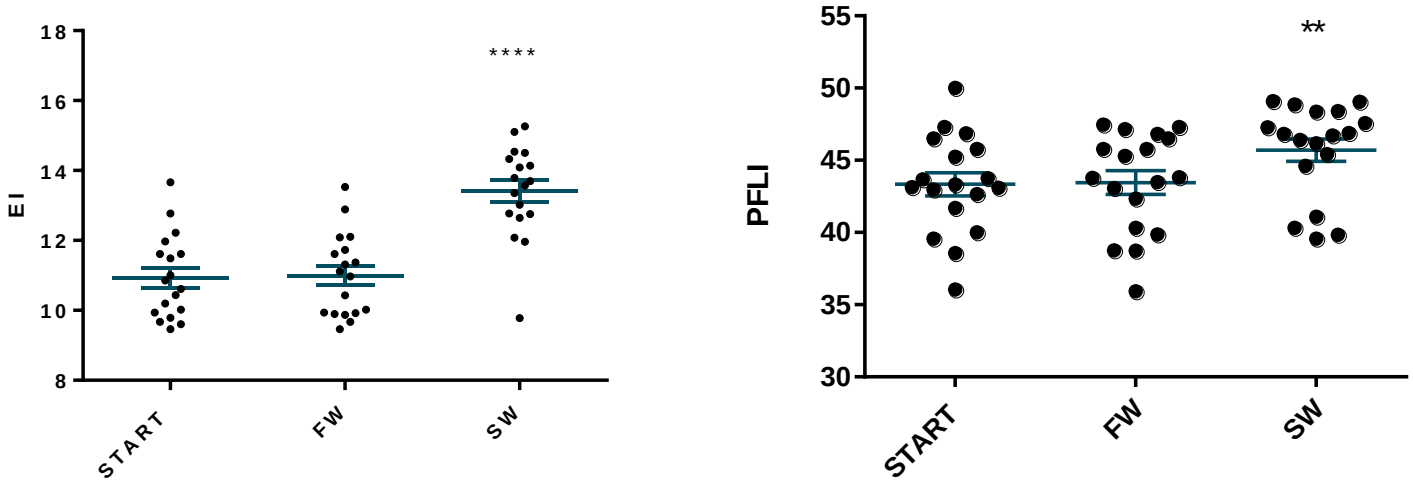

Figure 3. Eye Index (EI), Pectoral Fin Lenght Index (PFLI) and fat percentage of 18 experimental eels duringsimulated migration. Results are obtained via Paired, one tailed t-Student's Test and every month is compared with the previous one, errors bars represent SE. Asterisks bars indicate significant differences (* $p<0.05$; **$p<0.01$; ***$p<0.001$ and **** $p<0.0001$).

IIa. Maturatie en reproductie van oude kweekalen (exp. 1)

Randomly taken individual migrants of experiment 1 have been used in hormonal stimulation studies, injecting carp pituitary extract (CPE from 10 to 40 mg/kg; Mordenti et al., 2011) in natural seawater at 15 °C under dark conditions. 79% of the females (*n*=22 out of 28) matured: seventeen females matured after 12-20 weekly injections (GSI>30). When two days after injection the BWI exceeded 110%, eels were given another CPE injection and were transferred to a separate tank in seawater at 20 °C. One day later they were induced to spawn by injecting 17, 20 β-dihydroxy-4-pregnen-3-one (DHP) at 22 h. Five more females were sampled after 20 weekly injections and had GSI values between 9 and 16.

Males (*n*=50) were given a single 1,000 IU injection of human chorionic gonadotropin (hCG; Kahn et al., 1987), fully matured after 10 weeks and delivered highly motile sperm after another 1,000 IU hCG booster injection.

Induction of natural spawning was not successful as these females did not ovulate. Most females that were used for stripping the eggs ovulated between 14-20 h after DHP injection. Five females spawned most of their eggs in a single batch that was successfully used for fertilisation. Early cell divisions were mostly asymmetrical and in all cases no further development was observed within 24 h after fertilisation. Because feeding larvae were absent, feeds could not be tested experimentally.

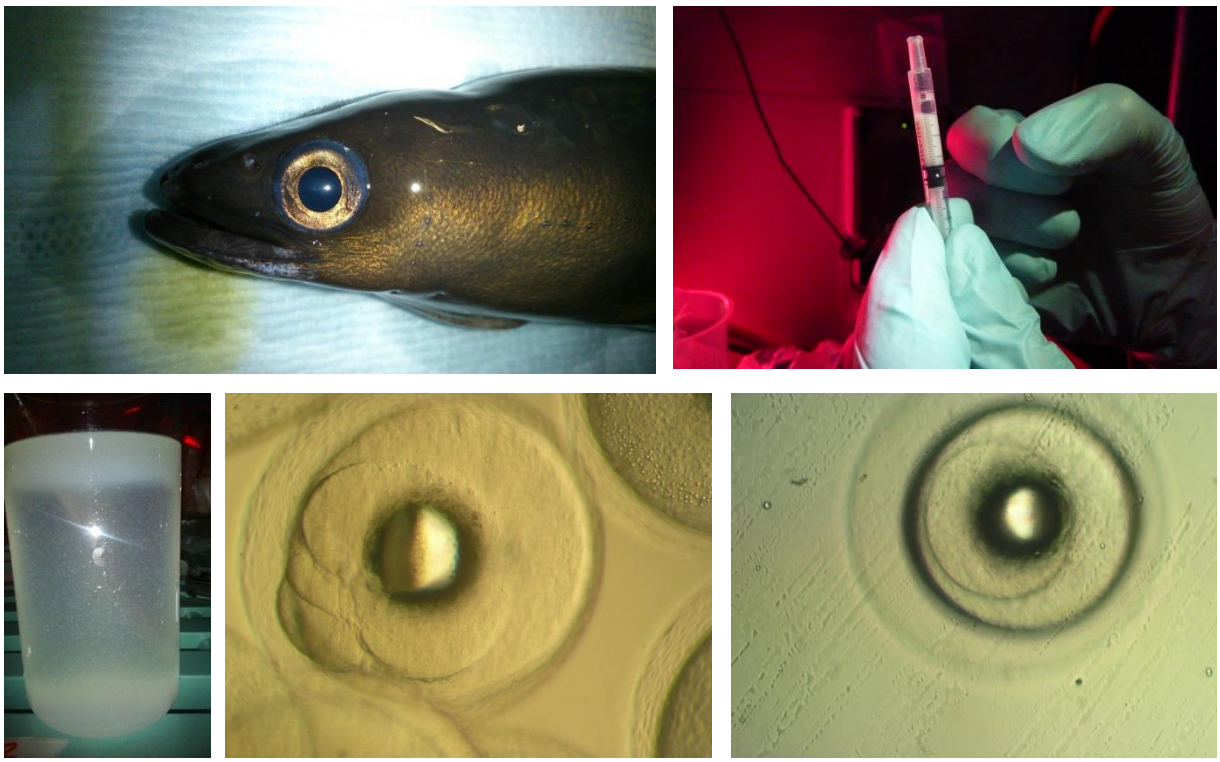

Figure 1. From top-left to bottom-right: mature female; stripped sperm; floating layer of fertilised eggs; early cell divisions.

1. References

Kahn IA, Lopez E., Leloup-Hatey E. (1987) Induction of spermatogenesis and spermiation by a single injection of Human Chorionic Gonadotropin in intact and hypophysectomized immature European eel (*Anguilla anguilla* L.). General and Comparative Endocrinology 68(1): 91-103.

Mordenti O, Di Biase A, Sirri R, Modugno S, Tasselli A (2011) Induction of sexual maturation in wild female European eels (*Anguilla anguilla*) in Darkness and Light. The Israeli Journal of Aquaculture - Bamidgeh, IJA_64.2012.726, 9 pages

IIb. Maturatie en reproductie van gefeminiseerde alen (exp. 2)

A similar reproduction experiment was performed with the feminised eels as with the farmed eels. Individual migrants of experiment 2 (N=33) were used for hormonal stimulation with CPE (same dose, same conditions). Many eels died with infections on the site of injection having considerable GSI values between 10 and 20. Only 21% of the females fully matured: seven females matured after 12-20 weekly injections (GSI>30).

Spermiating males (n=4) were provided by Glasaal Volendam.

Five females ovulated between 12-20 h after DHP injection and were used for stripping the eggs. Three of these eels were used for stripping another time a week later, again after booster injection and injection of DHP. Gamete quality was poor without any significant layer of floating eggs. Stripped eggs still contained many fat droplets (>10). Also now early cell divisions were mostly asymmetrical and in all cases no further development was observed within 24 h after fertilisation. Because feeding larvae were absent, feeds could not be tested experimentally.

Figure 1. From top-left to bottom-right: Erasmus guest worker Marco Graziano measuring fat percentage; a pregnant eel; the hatchery; MSc intern Thijs Böhm observing the eggs.

1. Conclusions

- FW migration increases plasma testosterone levels in both sexes but does not enhance sexual maturation;
- SW migration significantly increases gonad weight and GSI in both sexes but eye size only in males;
- The observed induced progression in maturation appears to be under steroid control;
- Simulation of migration under mimicked conditions may be used as a tool to condition farmed silver eels as broodstock in artificial reproduction trials;
- Feminised eels appear to be more sensitive to conditioning by simulated migration as these eels show significant eye enlargement while farmed eels do not;
- Eels that were subjected to simulated migration could be matured successfully: 17 farmed eels and 7 feminised eels fully matured, 10 egg batches were fertilised;
- Induction of spawning was not successful;
- No larvae could be obtained obstructing testing experimental larval feeds.

2. Disseminatie

Mes, D., Dirks, R.P., Palstra, A.P. (2014) Simulated migration under mimicked photothermal conditions enhances sexual maturation of European eel (*Anguilla anguilla*). 2nd FITFISH workshop on the Swimming Physiology of Fish, October 10, Barcelona (Spain)

Mes, D., Dirks, R.P., Palstra, A.P. (2015) Simulated migration under mimicked photothermal conditions enhances sexual maturation of European eel (*Anguilla anguilla*). AE2015, October 20-23, Rotterdam (Netherlands)

Mes, D., Dirks, R.P., Palstra, A.P. (2016) Simulated migration under mimicked photothermal conditions enhances sexual maturation of farmed European eel (*Anguilla anguilla*). Aquaculture 452: 367–372.

3. Publicity

- National newspaper NRC next (Nov 2013) Moeizame palingseks in Volendam
- Noord-Hollands Dagblad, Leidsch Dagblad (May 2015) Van glasaal tot schieraal
- Volkskrant bijlage V-zomer (Jul 2015) Ook kweekvissen hebben lichaamsbeweging nodig. Interview met A.P. Palstra
- WUR magazine Resource (Nov 2015) Zwemmen brengt palingen in de puberteit.
- België VRT radio 1 (Dec 2015) De liefdestrektocht van de paling.
- EOS magazine: Zwemmen doet paling puberen (Dec 2015)

Eel pitch for EU commissioner Karmenu Vella.

Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 187378-2015-AQ-NLD-RvA). This certificate is valid until 15 September 2018. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation. The scope can be found at the website of the Council for Accreditation (www.rva.nl).

On the basis of this accreditation, the quality characteristic Q is awarded to results of components which are incorporated in the scope, provided they comply with all quality requirements, as described in the applied Internal Standard Working procedure (ISW) of the relevant accredited test method.

The quality of the test methods is ensured in various ways. The accuracy of the analysis is regularly assessed by participation in inter-laboratory performance studies including those organized by QUASIMEME. If no inter-laboratory study is available, a second-level control is performed. In addition, a first-level control is performed for each series of measurements.
In addition to the line controls the following general quality controls are carried out:

- Blank research.
- Recovery.
- Internal standard
- Injection standard.
- Sensitivity.

The above controls are described in IMARES ISW 2.10.2.105.

If the quality cannot be guaranteed, appropriate measures are taken.

Justification

VIP Report C061/16
Project Number: 4304306101

The scientific quality of this report has been peer reviewed by a colleague scientist and by the head of the department of IMARES.

Approved: Ron Dirks Ph.D.
Colleague scientist

Signature: 

Date: 14 June 2016

Approved: Dr. Ir. T.P. Bult
Business Unit Manager

Signature: 

Date: 14 June 2016

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IMARES (Institute for Marine Resources and Ecosystem Studies) is the Netherlands research institute established to provide the scientific support that is essential for developing policies and innovation in respect of the marine environment, fishery activities, aquaculture and the maritime sector.

The IMARES vision
'To explore the potential of marine nature to improve the quality of life'

The IMARES mission
- To conduct research with the aim of acquiring knowledge and offering advice on the sustainable management and use of marine and coastal areas.
- IMARES is an independent, leading scientific research institute

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