Spawning Migration of the European Eel
FISH & FISHERIES SERIES

VOLUME 30

Series Editors: David L.G. Noakes, Fisheries & Wildlife Department, Oregon State University, Corvallis, USA

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J. Cliff Rankin
Editors

Spawnning Migration of the European Eel

Reproduction Index, a Useful Tool for Conservation Management
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Chapter 10
Effects of Swimming on Silvering and Maturation of the European Eel, *Anguilla anguilla* L.

Arjan Palstra, Vincent van Ginneken, and Guido van den Thillart

**Abbreviations** 11-KT: 11-ketotestosterone; ACTH: adrenocorticotropic hormone; Ca: calcium; DHP: dihydroxy progesterone (17, 20β-dihydroxy-4-pregnen-3-one); E2: 17β-estradiol; Ei: eye index; ERα: estradiol receptor α; FSH: follicle stimulating hormone; FSH-β: FSH specific subunit β; FW: fresh water; GnRH: gonadotropin releasing hormone; GPTα: gonadotropin common α subunit; GSI: gonadosomatic index (relative gonad mass); LH: luteinising hormone; LH-β: LH specific subunit β; NEFA: non-esterified fatty acids; q-rPCR: quantitative real time polymerase chain reaction; StAR: steroidogenic acute regulatory protein; SW: salt water; T: testosteron; VTG: vitellogenin; αMSH: melanophore-stimulating hormone α

10.1 Introduction

Every year at the end of the growth season in autumn, the majority of the large European eels cease feeding, become restless and start to mature. Only those with sufficient lipid stores (Larsson et al. 1990; Svedäng and Wickström 1997) will start their reproductive migration to the spawning grounds in the Sargasso. They leave in a prepubertal condition with less than 2% relative gonad mass (GSI). The smaller males leave by August (Usui 1991). The large and fatty females leave between October and December to arrive an estimated 3.5 months later (Palstra et al. 2008a) at the spawning grounds. There, in early spring, males reach gonad masses of 10% and females of 40% to 60% of the body mass (Palstra et al. 2005).

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G. van den Thillart et al. (eds.), *Spawning Migration of the European Eel*, 229
They are sexually mature and ready to spawn more than one million eggs (Van Ginneken et al. 2005).

As European eels have to swim about 5,500-km to reach their spawning site, swimming is supposed to play a crucial role in natural triggering and stimulation of maturation. Fine-tuning between migration and maturation of fishes is a non-elucidated research topic that deserves much more attention. It is peculiar that the influence of swim exercise on maturation has never been thoroughly investigated, especially since fatty migrant fishes like tuna and eel are of major commercial interest but very difficult or even impossible to reproduce in captivity.

10.2 Environmental Triggers of Silverying and Maturation

10.2.1 The Continental Phase: Depressed Lipid Mobilization and Pre-pubertal Blockage of Maturation

The continental phase in the life cycle of the European eel can be considered as a feeding phase to attain the lipid reserves to fuel migration and to provide the future offspring with sufficient energy. In order to store the required amount of lipid before maturing, this phase is characterised by a severely depressed lipid mobilization (EELREP 2005) and blockage of maturation. Pre-pubertal blockage of maturation is due to a deficient gonadotropin releasing hormone (GnRH) stimulation and a simultaneous inhibition of the pituitary gonadotropes FSH and LH by dopamine (Chapter 12). These gonadotropes control gonad development directly, or indirectly by acting on steroid metabolism in the production of estrogens and androgens in both male and female eels. This dual neuroendocrine control is extreme, but not specific for eels and occurs in various adult teleosts (Vidal et al. 2004). However, dopamine only counteracts regulation of the last steps of gametogenesis in these species while in eel, dopamine seems to play a role in earlier stages (Vidal et al. 2004). The extreme blockage of maturation is the main reason why eel still cannot be bred naturally in captivity.

10.2.2 Possible Environmental Triggers of Maturation

Temperature and light do not show a clear effect on the hypothalamo-pituitary-gonad axis in silver eels (Boëtius and Boëtius 1967; Nilsson et al. 1981). Salinity seems to play a role as trigger since GSI and oocyte diameters increased after rearing Japanese farmed eels for 3 months in seawater up to values that are similar for wild silver females (Kagawa et al. 1998; Kagawa 2003). Also water pressure has been demonstrated to have a positive effect on maturation (Dufour and Fontaine 1985), also suggested by the observation of a migrating silver eel with swollen belly at the Bahamas at 2,000-m depth (Robins et al. 1979). After 3 weeks
exposure of eels to high pressure in hyperbaric chambers, Sebert et al. (2005) found a decrease in thyroxin hydroxylase mRNA levels; the rate limiting enzyme in the biosynthesis of dopamine, suggesting that high pressure is involved in lifting the dopaminergic inhibition and triggering maturation. Recently, Sebert et al. (2007) indeed found that females submitted to high pressure of 101 ATA showed a significant increase in oocyte diameter and plasma levels of E2 and VTG, while both sexes showed increased plasma levels of 11-KT.

10.2.3 Is Swimming the Trigger for Lipid Mobilization and Maturation?

Silvering marks the start of lipid mobilization and maturation. It appears that silvering is not a true metamorphosis e.g. a marked and abrupt developmental change in the form or structure of an animal, but a mere initiation of maturation; the start of puberty (Chapter 3). Silvering is more flexible than generally presumed (Svedäng and Wickström 1997) and may well be arrested at various stages as occurs for Atlantic salmon Salmo salar (Mills 1989). Durif et al. (2005) identified such intermediate phases and found that they were correlated to migration. The most advanced stages of maturation we know are from migrating silver eels Anguilla spp. caught closest to the spawning grounds. Moreover, a negative correlation seems to exist between migration distance to the spawning grounds and GSI at the start of oceanic migration of the various Anguilla species (Todd 1981).

From this we could assume that lipid mobilization and early maturation are linked to migration and that swimming itself may be the natural trigger for these processes. Indeed, no change in lipid mobilization was found between yellow and silver eels from the same location without having swum (EELREP 2005). We therefore hypothesized that lipolysis becomes activated during and due to sustained swimming. Furthermore, we hypothesized that swimming triggers silvering, the start of maturation, but that blockage of more advanced stages; vitellogenesis, is likely required in order to allow the long spawning migration.

10.3 Evidence from Experimental Swim Trials: Swimming Induces Silvering and Maturation

10.3.1 Experimental Swim Trials

At Leiden University (The Netherlands), numerous swim trials have been performed in 22 Blazka-type calibrated swim-flumes described in detail by Van den Thillart et al. (2004), elucidating aspects of swim performance of eels (Chapters 8, 9 and 16) and swimming induced silvering and maturation (this chapter). In 2006, a new stream-gutter (Fig. 10.1) was built to allow group-wise swimming of males and females, expected to lead to lower stress levels that would have negative effects on maturation.
Fig. 10.1 (a) The new stream gutter at Leiden University to allow group-wise swimming with (b) the front fence of the female compartment, (c) the curve before the female compartment with a constructed curved fence to create a faster and linear water velocity profile, (d) one of the two SPECK pumps creating a stream, (e) the stream gutter running during the experiment under conditions of far-red 670-nm light (bandwidth 20-nm), (f) the recirculating filter system with precipitation filter, sump, protein skimmer and biological filter and (g) experimental design of the round-shaped stream gutter. To allow a large amount of eels, the straight ends were extended creating the shape of a racetrack. Two compartments allow swimming of males (top) and females (bottom). The female compartment is only half the width of the male compartment to create a two-fold harder stream
10.3.2 Swimming Induced Changes in External Appearance

Swimming induces an increase of eye diameter (Table 10.1). This has been observed repeatedly in different swim trials. Continuous swimming at 0.5 body-lengths per second of eels from the landlocked Lake Balaton resulted in an increase of the eye index (EI) that was already apparent after 2 weeks and occurred in all eels exposed to the swim trial (Palstra et al. 2007a). The observed changes appeared

Table 10.1 Experimental swim-trials performed at Leiden University in (a) fresh water (FW) and (b) salt water (SW)

<table>
<thead>
<tr>
<th>Origin</th>
<th>n</th>
<th>Sex (m/f)</th>
<th>Age (year)</th>
<th>Migr. period (weeks)</th>
<th>Migr. distance (km)</th>
<th>EI</th>
<th>Stage</th>
<th>Reference</th>
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<tr>
<td>(a) FW trials</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>River Loire (Fr)</td>
<td>20</td>
<td>f</td>
<td>16 ± 1</td>
<td>1</td>
<td>&lt;300</td>
<td>+</td>
<td>Stage 3</td>
<td>Palstra et al. 2008a</td>
</tr>
<tr>
<td>Lake Balaton</td>
<td>6</td>
<td>f</td>
<td>16 ± 1</td>
<td>2</td>
<td>350</td>
<td>+</td>
<td>Stage 3</td>
<td>Palstra et al. 2007a</td>
</tr>
<tr>
<td>Lake Balaton</td>
<td>9</td>
<td>f</td>
<td>16 ± 1</td>
<td>6</td>
<td>1,100</td>
<td>+</td>
<td>Stage 3</td>
<td>Palstra et al. 2007a</td>
</tr>
<tr>
<td>Farm</td>
<td>6</td>
<td>f</td>
<td>5</td>
<td>6</td>
<td>2,200</td>
<td>+</td>
<td>Stage 3</td>
<td>Palstra et al. 2006a</td>
</tr>
<tr>
<td>Farm</td>
<td>15</td>
<td>f</td>
<td>3</td>
<td>25</td>
<td>5,500</td>
<td>-</td>
<td>Stage 3</td>
<td>Van Ginneken et al. 2007a</td>
</tr>
<tr>
<td>(b) SW trials</td>
<td></td>
<td></td>
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<tr>
<td>Lake Grevelingen (NL)</td>
<td>20</td>
<td>f</td>
<td>11 ± 4</td>
<td>0.6</td>
<td>&lt;300</td>
<td>-</td>
<td>Stage 3</td>
<td>Palstra et al. 2008a</td>
</tr>
<tr>
<td>Lake Grevelingen (NL)</td>
<td>6</td>
<td>f</td>
<td>8 ± 2</td>
<td>4</td>
<td>1,200</td>
<td>-</td>
<td>Stage 3</td>
<td>Palstra et al. 2006a</td>
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<tr>
<td>Lake Grevelingen (NL)</td>
<td>11</td>
<td>f</td>
<td>4</td>
<td>1,000</td>
<td>-</td>
<td>Stage 3</td>
<td>A. Palstra et al., 2000, unpublished data</td>
<td></td>
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<tr>
<td>Greece</td>
<td>6</td>
<td>f</td>
<td>6</td>
<td>900</td>
<td>-</td>
<td>Stage 3</td>
<td>Palstra et al. 2007c</td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>6</td>
<td>f</td>
<td>12</td>
<td>1,400</td>
<td>-</td>
<td>Stage 3</td>
<td>Palstra et al. 2007c</td>
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</tr>
<tr>
<td>Greece</td>
<td>6</td>
<td>m</td>
<td>6</td>
<td>500</td>
<td>-</td>
<td>Stage 2</td>
<td>A. Palstra et al., 2007, unpublished data</td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>6</td>
<td>m</td>
<td>12</td>
<td>900</td>
<td>-</td>
<td>Stage 2</td>
<td>A. Palstra et al., 2007, unpublished data</td>
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Columns represent the origin of experimental eels, the number (n), their sex male (m) or female (f), their age in years, the experimental simulated migration period in weeks, the migrational distance in km, occurrence of changes in eye index EI (+) or not (−), the dominant gonad stage after swimming and the literature reference. The asterisk marks an estimated age corresponding with findings for other Lake Balaton eels.
even stronger after 6 weeks of swimming. Significant increases were also apparent in swim-trials with migrating eels from the River Loire and with older farmed eels (Table 10.1). Since younger farmed eels did not show swimming – induced increase of the eyes, age – dependent maturation sensitivity is suggested (see later in this chapter). It also appears that this phenomenon solely occurs in fresh-water since no changes were detected in salt-water trials (Table 10.1), in either males or females.

Since the enlargement of the eyes is used for discriminating between the yellow and silver phase (Pankhurst 1982), it can thus be stated that swimming induces silvering. Changes in the length (Durif et al. 2005) and shape (Tesch 2003) of the pectoral fins are also considered as indicative of the degree of silvering. However, such changes were not detected in any of the swim-trials (Palstra et al. 2007a).

### 10.3.3 Swimming Induced Changes in Oocyte Histology

The ovaries of European silver eels show oocytes after transformation of the oogonia in the first developmental stages (stage 1–2; Adachi et al. 2003). Further progression requires incorporation of lipids (stage 3) and vitellogenin (stage 4). Already after 1 week of swimming of Lake Balaton eels (Palstra et al. 2007a), the GSI increased and oocytes became larger with large numbers of lipid droplets (Figs. 10.2 and 10.3). After 6 weeks of swimming, changes were much more pronounced than after 2 weeks of swimming, both GSI and oocyte diameter were significantly higher. In contrast to resting eels the swimming eels had oocytes in the lipid droplet stage 3. These results indicate that a high level of lipid mobilization induced by swimming is required not only for fuel but also for a natural incorporation of lipid droplets in the oocytes. Oocytes of eels that had swum contained more than 100 large droplets. Most developed oocytes had lipid droplets that covered >50% of the cytoplasm and formed a complete ring around the circumference of the developing oocyte (Couillard et al. 1997), which is typical for previtellogenic oocytes (Colombo et al. 1984).

However, we did not observe any yolk globuli in the oocytes. Also the oocytes did not reach sizes that are characteristic for vitellogenesis (stage 4). Adachi et al. (2003) showed for A. japonica that vitellogenesis begins when oocytes are about 250μm in diameter. In our studies we found maximum oocyte diameters of 236μm, which are quite close to the onset of vitellogenesis.

### 10.3.4 Swimming – Induced Changes in Testis Histology

Male European silver eels have a GSI <0.2%. Swimming male eels had a higher GSI after 1.5 months swimming and even higher after 3 months swimming (Fig. 10.4; Palstra et al., 2008b). These changes in GSI reflected histological changes in testis development and the onset of spermatogenesis. The testes of European silver eels show thick layers of connective tissue. Spermatogonial stem cells are present with clear large homogenous nuclei containing one or two nucleoli (Fig. 10.4). Spermatogonia occur independently (type A) or in cysts of two or four germ cells (type B; Miura et al. 2003). Swimming male eels had higher percentages of stage 2 and late
Fig. 10.2 Oocyte development in swimming eels, representing subsequent stages in (a) a Lake Balaton eel before swimming with stage 1 and 2 oocytes, (b) a Lake Balaton eel after 2 weeks swimming (350-km) with many stage 3 oocytes but also still less developed oocytes, (c) a Lake Balaton eel after 6 weeks swimming (1,100-km) with mainly stage 3 oocytes with many lipid droplets and decreasing lipid stores, (d) a 5 year old farmed eel with mainly early stage 3 oocytes, (e) a 5 year old farmed eel after 2,200-km of swimming with solely stage 3 oocytes, (f) a migrating silver eel from Lake Grevelingen with stage 3 oocytes and fat reserves, (g) a migrating silver eel from Lake Grevelingen with stage 300 oocytes and fat reserves, (g) a silver eel from Lake Grevelingen after swimming 1,200-km with stage 3 oocytes fully covered with large lipid droplets, and (h) an artificially matured Lake Grevelingen eel with oocytes containing yolk globuli during final maturation. Swimming stimulates incorporation of large amounts of lipid and synchronizes development up to late stage 3 oocytes. The scale bar represent 100μm; the scale bar in a accounts for a to g, h is on a smaller scale.

Fig. 10.3 Histological differences in oocyte development between Lake Balaton eels that rested or swam for 2 weeks in (a) oocyte diameter, (b) the number of lipid droplets in the oocytes and (c) the diameter of lipid droplets (Based on data from Palstra et al. 2007a)
Fig. 10.4 Relative gonad mass (mean GSI + standard error) and testis histology in male eels. Experimental groups were sampled after swimming or resting after 1.5 and 3 months. The asterisk indicates a significant difference (P < 0.05) in GSI after 3 months swimming. Pictures from top to bottom show testis histology of a resting eel with stage 1 spermatogonia (top), a swimming eel for 1.5 months with a GSI = 0.32 with clear clusters of stage 2 spermatogonia (middle) and a swimming eel for 3 months with a GSI = 0.59 with stage 3 spermatocytes (bottom). The scale bar represent 100 μm.

type b spermatogonia (Fig. 10.4). An increase of organization in clusters occurred with spermatogonia grouped in spermatid tubules, and a strong reduction of connective tissue and lipid tissue. The testis of one male eel that swam for 3 months even contained spermatocytes. Our latest results show that male eels that swam for 3 months in salt water and that were subsequently treated with human chorionic gonadotropin started their spermiation earlier, and they produced more sperm of higher density.

10.3.5 Swimming – Induced Changes in Pituitary and Blood Plasma Maturation Parameters

After swimming 5,500-km in fresh-water, young farmed eels showed increased LH levels in the pituitary (Fig. 10.5; Van Ginneken et al. 2007a). The same occurred in wild migratory eels from Lake Grevelingen after swimming 1,000-km in salt water (A. Palstra et al., 2000, unpublished data). Swimming thus stimulates gonadotrope production of the pituitary but it is still unclear whether secretion is also stimulated.
After swimming 5,500-km in fresh-water, the young farmed eels also showed a tendency to an increased level of plasma 11-KT (Fig. 10.5), while plasma-VTG, pituitary-adrenocorticotropic hormone (ACTH), plasma-ACTH, pituitary-melanophore-stimulating hormone (MSH) α and plasma-αMSH were unaffected (Van Ginneken et al. 2007a). 11-KT is considered as the major hormonal mediator of silvering in female eels (Lokman et al. 2003), as evidenced by the numerous silvering effects, like enlargement of the eyes, that Rohr et al. (2001) observed after implanting 11-KT in female A. australis. Just recently, Endo et al. (2007) showed that 11-KT has an important role in lipid transfer and deposition in the oocytes which agrees with the histological observations. The young farmed eels in Van Ginneken’s study however did not show increase in eye size but other swimming eels did after swimming in fresh-water (Table 10.1).

VTG levels in swimming Lake Balaton eels were below detection limits (A. Palstra et al. 2005, unpublished data). Wild migratory silver eels even showed decreased levels of plasma VTG after swimming 1,000-km in salt water while testosterone (T) and E2 were unaffected (A. Palstra et al. 2005, unpublished data). Recently, we measured blood plasma E2 and VTG indirectly through plasma calcium (Ca) (Palstra et al. 2007c). A significant positive correlation and similar sensitivity to VTG has been demonstrated for rainbow trout, Oncorhynchus mykiss, by Verslycke et al. (2002) and used on eels by Versonnen et al. (2004). Swimming, but also resting, increased E2 levels but only in first instance. Ca levels were found lower in swimming eels. Results thus show that swimming does not stimulate vitellogenesis which corresponds with histological findings; the absence of yolk globuli in the oocytes of swimmers.
10.3.6 Swimming - Induced Changes in Expression Profiles of Maturation Parameters

Recently, we have cloned four different genes from eel tissue extracts (Palstra et al. 2007c): E2 receptor α (ERα), VTG 1, VTG 2 and β-actin. We have applied the developed molecular probes for housekeeping gene β-actin and targeted genes for ERα, VTG 1- and VTG 2-expression on liver samples of female silver eels that swam for 1.5 or 3 months in salt water. In swimming eels, the expression of ERα was slightly lower and in resting eels higher than in the control group (Fig. 10.6a). This reduction in expression probably caused the reduced expression of VTG 1 and VTG 2. From this we can conclude that hepatic vitellogenesis is indeed reduced in swimming silver eels in salt water.

FSH and LH play separate roles (Suetake et al. 2003) during maturation of teleost fishes. In general, FSH is related to early maturation (vitellogenesis/spermatogenesis) and LH related with late maturation processes (maturation/spermiation). During artificially induced maturation, an immediate FSH-β decrease and a LH-β over-expression occur in female Japanese (Nagae et al. 1993; Saito et al. 2003; Suetake et al. 2002) and European eels (Schmitz et al. 2005). Their relatives, naturally maturing New Zealand longfinned eels Anguilla dieffenbachii (Suetake et al. 2002) and common Japanese congers (Suetake et al. 2003), however show high FSHβ expression levels suggesting that this decrease is abnormal. The question is now whether a natural stimulator of maturation, like swimming, causes an increase in FSH expression. At this moment we are investigating the expression of the common α-subunit (GPRα) and the specific β-subunits (FSH-β and LH-β) in the pituitary and the expression of their receptors in the gonads of swimmers. The latest results (Palstra et al. 2008b) have shown that swimming did not have a significant effect on FSHβ expression. Swimming did cause a two- to three-fold higher LHB expression but only in males and not in females.

10.4 Hypothesis for a Mechanism

10.4.1 Swimming Activates Lipid Metabolism

Swimming eels were found to have large oocytes in the lipid droplet stage containing large amounts of lipid droplets (Palstra et al. 2007a). These results indicate that a high level of lipid mobilization induced by swimming is required not only to fuel migration but also for a natural incorporation of lipid droplets in the oocytes. This is regulated by swimming increased 11-KT levels and represents a crucial step in oocyte maturation since the amount of lipid droplets influences the following developmental events before and after fertilization (Palstra et al. 2005), and provides the necessary reserves for the offspring. For comparison, in artificially matured eels, 57 ± 22 g lipid is incorporated into the oocytes corresponding to 28% of the lipid reserves of the average silver eel (Palstra et al. 2006a).
Fig. 10.6  Ercc-, VTG 1- and VTG 2-expression (mean number of copies of mRNA per ngram of total RNA with standard error) in livers after (a) resting or swimming for 1.5 and 3 months, and (b) of 1.5 and 3 months rest and swim groups that were subsequently stimulated with three weekly CPE injections. Asterisks indicate a significant difference (P < 0.05) of a rest group vs. the control (Based on data from Palstra et al. 2007c)
10.4.2 Swimming Inhibits Vitellogenesis

On the basis of evidence from different angles, it can be concluded that swimming inhibits the whole process of vitellogenesis, at least in first instance. Firstly, ERTc, VTG 1- and VTG 2-expression were reduced in the livers of swimming females (Palstra et al. 2007c). Secondly, plasma VTG and Ca were repeatedly determined as not detectable and not elevated in swimming females (Van Ginneken et al. 2007a, Palstra et al. 2007c). Thirdly, oocytes of swimming females from Lake Balaton (Palstra et al. 2007a), Lake Grevelingen (A. Palstra et al., 2002, unpublished) and River Loire (A. Palstra et al., 2004, unpublished) did not contain any yolk globuli and were all smaller than 250 μm, the border for switching to stage 4 vitellogenic oocytes.

10.4.3 Linking Metabolism with Maturation: A Central Role for Cortisol

We hypothesize that cortisol plays a major role in the endocrinological connection between metabolism and maturation. Silver eels have higher cortisol levels (Van Ginneken et al. 2007b) and higher cortisol levels have been measured in swimming eels of Lake Grevelingen (A. Palstra et al., 2000, unpublished data) and Lake Balaton although individual variance is very high (Fig. 10.7). Cortisol is known for mobilization of lipids. Cortisol peaks lead to lysis of muscle and hepatic lipids (Freeman and Idler 1973; Davis et al. 1985; Barton et al. 1987; Mommsen et al. 1999) releasing fatty acids into the blood.

![Cortisol levels](image)

**Fig. 10.7** Individual percentual change (%) in cortisol levels before and after swimming for (a) 2 weeks and (b) 6 weeks of Lake Balaton eels. After swimming 2 weeks, only one eel showed increase of cortisol level (>200%). After swimming 6 weeks, six out of eight eels showed increase of cortisol level with three eels showing an increase >200%.
Cortisol has a less defined role in maturation of fishes. DiBattista et al. (2005) showed that cortisol treatment in rainbow trout significantly decreased dopaminergic activity in the telencephalon. Epstein et al. (1971) and Fontaine (1994) showed that successive high concentrations of plasma cortisol, lasting for at least 7 days, triggers silverying in the eel. Cortisol is also known as stimulator of LH synthesis in vitro and in vivo (Huang et al. 1999; Dufour et al. 2003) and as inhibitor of vitellogenin synthesis (Sbaihi et al. 2001). Cortisol was shown to inhibit E2-induced vitellogenin synthesis in the rainbow trout, an effect mediated by a decrease in ERα mRNA levels (Lethimonier-Desdoits et al. 2000). These observations perfectly match our observations on swimming eels.

Swimming increases cortisol levels chronically and with this, key steps in steroid metabolism may be stimulated. By activating lipid metabolism, cortisol may increase cholesterol transport in plasma by lipoproteins. In the cell, cholesterol is transported from the outer to the inner mitochondrial membrane by steriodogenic acute regulatory protein STAR (Li et al. 2003) and then transformed into pregnenolone by cytochrome P-450 cholesterol sidechain cleavage enzyme; the rate-limiting step (Stocco 2001) before transformations into for instance 11-ketotestosteron, estradiol and dihydroxy progesterone (DHP). Recently, Kazeto et al. (2006) found that transcript levels of this enzyme increased in the ovary of Japanese eels during artificially induced ovarian development, suggesting that expression is induced by gonadotrope stimuli. Precursor 17α hydroxyprogesteron can be transformed back into cortisol creating a possible regulatory mechanism.

10.5 The Necessity of Swimming for Reproduction

10.5.1 Age Dependent Sensitivity for Maturation

Otolith analysis has revealed the old age of Lake Balaton eels (13–21 years) and migratory Loire eels (10–28 years) at the time of experimenting (Palstra et al. 2008a, 2006a, 2007a). These eels show extensive changes after just 1 week swimming while young, 3 year old, farmed eels only showed minor changes under identical conditions (Table 10.1). These results suggest that older eels are more sensitive to stimulation of maturation and that maturation is an age–dependent process (Palstra et al. 2007a). Indeed, older farmed eels did show increase of the eyes within 1 week swimming (Table 10.1). Arguments for age–dependent maturation also come from other observations: (1) older eels showed increased capacity to incorporate more lipid from the muscle into the oocytes (Palstra et al. 2006b), and (2) older eels were more sensitive to hormonal stimulation (Palstra et al. 2006a; also Durif et al. 2006). Repeated yearly silverying and subsequent regression (Durif et al. 2005) might unlock the strong inhibition of sexual maturation in eels. Age might thus be a key factor for successful maturation.
10.5.2 Does Swimming Increase the Maturation Sensitivity?

In a recent study (Palstra et al. 2007c), we have tried to find time points during swimming where maturation sensitivity increases. Groups of females that had either swum or rested for 1.5 months and for 3 months were subjected to a maturation sensitivity test (EELREP 2005; Durif et al. 2006). In this test, eels were injected weekly IP with 20-mg carp pituitary extract for a period of 3 weeks since at this point eels show elevated expression of VTG 1 and VTG 2, elevated plasma calcium levels and yolk globuli appearing in the oocytes (A. Palstra et al., 2005, unpublished data). In this study, blood plasma E2 and Ca levels increased when swimming and resting eels were subsequently stimulated by three weekly CPE injections. Expression of ERα-, VTG 1- and VTG 2-strongly increased after hormonal stimulation. Female eels that has swim for three months did however not show a higher sensitivity (Fig. 10.6b). Recently (A. Palstra et al., 2007, unpublished results) we even found that swimmers evaluated on average 2-3 weeks later than the resters. In contrast, male swimmers were more sensitive and spermated earlier in response to hormonal treatment than resters.

10.5.3 Does Swimming Increase Gamete Quality?

Although current protocols for artificial reproduction of eels by hormonal injections are successful to a certain extent (Kagawa et al. 2005; Chapter 15), the induced process of maturation can be considered abnormal in many aspects. Abnormality of maturation is evidenced by limited reproductive success (Pedersen 2003, 2004; Palstra et al. 2005 and references therein), and observed phenomena like variations in yolk accumulation, egg membrane formation, differences in the process of oocyte maturation and plasma hormone levels (Adachi et al. 2003; Kagawa et al. 2005). Oocytes of non-exercised silver eels are probably still too premature for hormonal stimulation by pituitary extract. Two probable causes of abnormality may be prevented by swimming: (1) incomplete lipid incorporation, and (2) incomplete vitellogenesis.

As for point 1, during artificial induction by hormonal injections, hepatic vitellogenesis is immediately induced (A. Palstra et al., 2005, unpublished data) Lipid and VTG incorporation occur simultaneously in artificially matured Japanese eel (Adachi et al. 2003) and European eel (A. Palstra et al., 2005, unpublished data), which suggests an unnatural situation. By stimulating incorporation of lipids in the oocytes and inhibiting vitellogenesis, swimming may optimize the natural sequence of these processes.

As for point 2, the effects of FSH in European eel are still largely unclear. Plasma FSH levels are higher in migrating silver eels (Dufour et al. 2003) indicating a relation with swimming. Kamei et al. (2005, 2006) recently showed for Japanese eel that FSH stimulated in vitro: (1) T and 11-KT secretions in a dose-dependent manner in immature testis, and (2) T and E2 secretion in a dose-dependent manner from mid-vitellogenic oocytes. Early oocyte stages lacked fully developed
theca and granulosa cells and respective secretion of T and E2. The action of both T and E2 may be required for hepatic vitellogenin synthesis (Kwon et al. 2005). Vitellogenesis through administration of solely estrogens to silver eels has never succeeded (Oliverseau and Oliverseau 1979; Petersen and Korsgaard 1989; Peyon et al. 1993 as reviewed by Lokman et al. 2003). As stated before, artificially induced maturation may lack FSH effects and the observed phenomena of abnormal maturation may well be consequences of a lack of (swimming increased) FSH. Sato et al. (2003) reported that long term treatment of FSH (21 to 23 weekly injections) followed by weekly LH injection lead to rapid maturation of oocytes that ovulated as eggs of the highest quality with highest fertilization rates. Long term swimming may induce such FSH effects and may, if followed by LH treatment, reflect the natural variant of these results.

Recently, Patterson et al. (2004) recognized this issue also for salmon and stated that "currently, exercise associated with migration is presented as an obstacle to successful reproduction. There has been no attempt, however, to reverse this paradigm and examine exercise as an integral part of normal reproductive development for long distance migrants". Indeed they found that non-exercised females had delayed maturity, lower egg deposition rates, and were more likely to die prior to egg ovulation than exercised females and natal spawners. Pre-treatment by swimming in current protocols for artificial reproduction will likely result in higher gamete quality and general reproductive success. These are topics of our current investigations.

10.6 Extrapolation to the Field

10.6.1 Fresh Water Migration: Silvering and Lipid Mobilization

When we ignore effects of other possible triggers and results from the laboratory are extrapolated to the field situation, it can be assumed that migratory eels do not necessarily silver before, but especially during, their fresh-water migration. Old swimming silver eels showed increases of eye size but it appears that this occurs only in fresh-water trials (Table 10.1). As far as we know, it is unknown if eels from salt or brackish water generally have larger eyes than eels from fresh water so that only the last mentioned show this swimming-induced change in eye size.

During fresh water migration, lipid mobilization occurs. Extensive lipid incorporation in the oocytes was apparent during fresh water swim-trials. In the field, Cottril et al. (2001) found that the concentration of total plasma non-esterified fatty acids (NEFA) appeared to follow the trend of E2 and GSI increasing with sexual maturity in migrating A. rostrata.

Vitellogenesis seems to be inhibited during fresh water migration. VTG and Ca levels are low in wild silver eels. Versonnen et al. (2004) measured Ca as indicator for VTG levels at 20 locations and found very low levels. In a recent study (A. Palstra et al., 2005, unpublished data), we measured E2 and Ca in blood plasma in migrating silver eels in the River Rhine (Germany) in August, September and
October 2005 and investigated the gonads histologically. E2 levels were higher in October but Ca levels stayed low over the months and oocytes were still smaller than 250μm without yolk globuli. A. Palstra et al., 2000, unpublished data measured VTG in 104 large female silver eels from the brackish Lake Grevelingen (The Netherlands). Of these eels, 96% showed low VTG levels <0.5μg ml⁻¹.

10.6.2 Oceanic Migration: Inhibiting Vitellogenesis

Salt water swim trials revealed no changes in eye diameter (Table 10.1). The question remains whether silver eels migrating in the ocean continue the increase in eye size. Probably, eyes will increase during progressive maturation since the eye diameter of silver eels from brackish waters increases further when stimulated by hormonal injections (Palstra 2006).

Also during oceanic migration, or at least during the first part, vitellogenesis seems to be reduced in migrating silver eels since eels did not show an increase in GSI and still showed reduced ERα-, VTG 1- and VTG 2-expression after swimming 3 months in salt water. This may be because (1) lifting the severely depressed lipid mobilization and extensive lipid incorporation requires long-term swim exercise, and (2) preventing undesired effects of vitellogenesis during swimming. VTG synthesis is associated with mobilization of phospho-calcium reserves coming from skeletal vertebral resorption (Shaihi 2002; Shaihi et al. 2007) which is naturally undesired during swimming. Growth of oocytes is most pronounced during vitellogenesis and subsequent maturation. The oocyte diameter will increase two-fold up to 400μm during vitellogenesis and again two-fold up to 800μm due to hydration during final maturation (Palstra et al. 2005). These increases result in similar increases of gonad mass and with that of the body diameter. This then increases drag during swimming and with that increases the cost of transport (reviewed by Van Ginneken and Maes 2005). Also this situation is considered as undesirable during migration.

10.6.3 Vitellogenesis and Maturation at the Spawning Grounds?

When we extrapolate lab results to the field, it can be hypothesized that vitellogenesis and maturation occur near or at the spawning grounds. Besides other triggers that were discussed before, spawning ground specific triggers may be involved during vitellogenesis and final maturation. Spawning ground specific triggering of the final stages of maturation has also been considered for other homing fishes (Palstra et al. 2004). These triggers may involve area-specific odour, the intersex pheromonal communication (Liley and Stacey 1983; Lam 1983; Van Ginneken et al. 2005; Huertas et al. 2006) or triggering by an increase in water temperature by rising in the water.
column. A rise in water temperature is known to increase the responsiveness of the liver to estrogen in the production of vitellogenins (Yaran et al. 1980).

10.7 Conclusions

During their continental phase, yellow eels have depressed lipid mobilization and pre-pubertal blockage of maturation (Fig. 10.8). Since the start of spawning migration marks the onset of lipid mobilization and maturation, swimming may be a crucial trigger of these processes. Swim trials of older eels in fresh water show that eyes were enlarged in all swimmers and that swimming thus induced silvering. Swimming stimulates early oocyte development and deposition of lipids in the oocytes of female eels (Fig. 10.8), most probably regulated by increased 11-ketotestosterone (11-KT) levels. Effects appear stronger in males where swimming induces spermatogenesis. Increased LH levels in the pituitary show that swimming stimulates its gonadotrope production. Swimming also increases plasma 17\(^\beta\)-estradiol (E2) but hepatic vitellogenesis is however not initiated: plasma vitellogenin (VTG) levels remain low and yolk globuli do not appear in the oocytes (Fig. 10.8). Swim trials of silver eels in salt water show that swimming inhibits vitellogenesis; expression of the E2 receptor \(\alpha\) (ER\(\alpha\)), VTG 1 and VTG 2 were lower in the liver of swimmers and plasma VTG levels were reduced. Lipid mobilization by swimming for fuel and deposition in the oocytes occurs most probably through the action of cortisol (Fig. 10.8) that is well-known as activator of lipid mobilization and has numerous positive effects on maturation parameters. Both the induction of lipid deposition in the oocytes and the obstruction of vitellogenesis by swimming may allow a natural sequence of events leading to a higher gamete quality in contrast to stimulation with pituitary extract injections. Such a natural sequence may reflect long term swimming – induced FSH effects followed by short term LH effects to finish maturation. The effect of long term swimming on gamete quality is subject of our current research. When these results are extrapolated to the field and linked to observations in the field, it appears that fresh water migration triggers silvering and lipid mobilization. Vitellogenesis is inhibited by swimming and probably occurs only near or at the spawning grounds. Our latest results show that male swimmers were more sensitive and spermiated earlier in response to hormonal treatment than resters.

Acknowledgements The authors would like to thank Prof. H. Spaink, Drs. D. Schnabel, C. Székely, C. Durić, F. Daverat, J. Klein-Breteler, G. Van der Laak, Ing. M. Nieveen, Ing. M. de Bakker, P. Niemantsverdriet, M. Fekkes, W. Spoore, E. Antonissen, M. Casteijn, R. Van der Linden, R. Heijmans, E. de Kuyper, J. Bij, M. Britijn, S. Van Schis, D. Curiel, J. Clavero, J. Van Rijsseb and M.A. Guerrero. The LU research was funded by the Dutch Technology Foundation (STW-project #LBI6.4199), the European Union (BIOREP #Q5RS-2001-01836) and the Dutch Ministry of Agriculture, Nature and Food Quality (project #320187).
Fig. 10.8 Model for swimming as trigger and stimulator of maturation and lipid incorporation with (a) During their continental feeding stage, maturation of eels is blocked at a pre-pubertal stage due to a deficient ghrelin stimulation and a simultaneous inhibition of the pituitary gonadotropes FSH and LH by dopamine (Dufour et al. 2003), and (b) During their fresh-water and oceanic reproductive migration, swimming lifts the dopaminergic inhibition probably through the action of cortisol. Cortisol mobilises lipid stores for fuel and for incorporation in the oocytes. Swimming induces oocyte development and lipid deposition during an extensive lipid droplet stage. Vitellogenesis is inhibited by swimming in first instance. Includes illustration from Palstra et al. 2007a (Reproduced from Palstra et al. 2007a. With permission of Elsevier)
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Chapter 11
The Gonadoliberin(S)-Gonadotropin(S) Axis in the Eel: Expression and Regulation Under Induced Maturation and Sex Steroid Feedbacks

Salima Aroua, Karine Rousseau, Monika Schmitz, Ching-Fong Chang, and Sylvie Dufour


11.1 Introduction

At the start of the reproductive migration towards the Sargasso Sea, silver eels are still immature and remain blocked at this prepubertal stage as long as migration is prevented. To date, adult mature eels have never been caught and only experimental treatments of silver eels with gonadotropic hormones have led to the observation of sexually mature animals (Fontaine 1936; Fontaine et al. 1964). The lack of sexual maturation at the silver stage is due to a deficient production of pituitary gonadotropins (GtHs) (Dufour et al. 1983a, b). Stimulation of synthesis and release of pituitary
gonadotropin in the silver eel can be induced by combined treatments with a GnRH-agonist and a dopamine-antagonist, indicating that a double neuroendocrine block was responsible for the deficient pituitary gonadotropic function: a lack of endogenous stimulation by GnRH due to a deficient production of GnRH and a strong dopaminergic inhibition of GnRH action (Dufour et al. 1988, 1991; Vidal et al. 2004).

In vertebrates, it is well known that the gonadotropins (luteinizing hormone, LH and follicle stimulating hormone, FSH) are secreted by the gonadotrophs in the anterior pituitary under the control of the gonadoliberin (GnRH) produced by the brain. The gonadotropins act on the ovaries and testes to promote gametogenesis and reproductive function, and to stimulate the production of sex steroids. In turn, gonadotropin secretion and subunit gene expression are regulated by sex steroids acting either directly on the gonadotrophs or indirectly by alterations of GnRH from the hypothalamus (Gharib et al. 1990). This review will mainly focus on the differential feedbacks exerted by sex steroids on the gonadoliberins/gonadotropins system in teleost fishes (especially the European eel).

### 11.2 GnRH System

To date, a total of 24 forms of GnRH have been isolated, 14 in vertebrates (12 in gnathostomes and 2 in lampreys) and 10 in invertebrates (9 in tunicates and 1 in molluscs) (for review: Gorbman and Sower 2003; Tsai 2006). Although GnRH peptides have not been isolated and sequenced from invertebrate neural structures, their presence has been suggested in cnidarians (for review: Rastogi et al. 2002; Gorbman and Sower 2003; Tsai 2006; Twan et al. 2006). In common with other neuropeptides GnRH peptides are first synthesized as a large precursor (pre-proGnRH), which includes a signal peptide (around 20–25 residues), the biologically active decapptide GnRH, a cleavage tripeptide (Gly-Lys-Arg) and the GnRH-associated Peptide (GAP; around 40–50 residues) (for review: Somoza et al. 2002; Lethimonier et al. 2004).

The majority of gnathostomes possess two forms of GnRH: the form in the preoptic-hypothalamic system is species-specific and highly variable, while the

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form in the hindbrain is consistently the chicken II form (cGnRH-II), considered as an ancestral peptide (Muske 1993). The highest diversity has been observed in teleosts (eight GnRHs isolated) (Table 11.1; Fig. 11.1). In early teleosts such as the eel (Elopomorphs), chicken GnRH-II coexists with mammalian GnRH (mGnRH) (Japanese eel Anguilla japonica; Nozaki et al. 1985; European eel Anguilla anguilla: King et al. 1990), a situation similar to that found in other primitive Actinopterygii (for instance in Chondrostei such as sturgeon). In most other teleosts, cGnRH-II coexists with salmon GnRH (sGnRH) (for instance: Salmoniforms, Cypriniforms and Osteoglossiforms), with the exception of Siluriforms where catfish GnRH (cfGnRH) is present. In recent teleosts, a third GnRH form (perciforms and rockfish: sbGnRH; medaka: mdGnRH = pejerrey: pGnRH) coexists with cGnRH-II and sGnRH. Interestingly, in more primitive species, the herring (clupeiform) and the whitefish (salmoniform), a third form was also identified (herring: hrGnRH; whitefish: wfGnRH), which also coexists with cGnRH-II and sGnRH.

In the European eel, a first immunocytochemical study of the brain distribution of GnRH neurons has been performed using antibodies recognizing all forms of GnRH (Kah et al. 1989). Two GnRH molecular forms, similar to mammalian GnRH (mGnRH) and to chicken GnRH-II (cGnRH-II), were then demonstrated by High Performance Liquid Chromatography (HPLC) and specific radioimmunoassay (RIA) (King et al. 1990). A differential distribution of these two forms was shown in the brain and the pituitary of silver eels (RIA using specific antisera: Dufour et al. 1993; immunocytochemistry: Montero et al. 1994). By RIA, we first showed that mGnRH levels were higher than cGnRH-II levels in the pituitary, olfactory lobes and tel-, di- and mes-encephalon, while the opposite was found in the posterior part of the brain (met- and myel-encephalon) (Dufour et al. 1993). These distributions were confirmed by our immunocytochemistry study in which mGnRH and cGnRH-II appeared to be produced by distinct neurons: mGnRH by neurons from olfactory bulbs, the nucleus olfactoretinalis, the ventral telencephalon, the preoptic
area and the hypothalamus, while cGnRH-II was produced in a few neurons in the midbrain tegmentum (Montero et al. 1994) (Fig. 11.2). All these data suggest differential physiological roles for the two GnRH forms in the eel. The occurrence of two GnRH forms in the eel brain was confirmed by the isolation of their cDNAs and genes in the Japanese eel (Okubo et al. 1999a, b). In addition, this study demonstrated the occurrence of three splicing variants of the messenger RNA coding for mGnRH, revealing further diversity of mGnRH potential roles and regulation (Okubo et al. 1999a, b). We are currently investigating the distribution and regulation of mGnRH and cGnRH-II precursors in the European eel (Weltzien, Dufour and coworkers, in the frame of the Norway-France international cooperation).

The mGnRH molecular form, as found in the eel, is thought to have first appeared in evolution in early-emerged bony fish (Osteichthyes) based on evidence from two species of Chondrostei (reelfish and sturgeon), one species of Pinguimodii (gar) and one species of Halecomorphi (Amia) (for review: O’Neill et al. 1998), as well as two species of primitive Teleosteii (eels and butterfly fish). The question of the existence of an undiscovered third GnRH form in the eel, in addition to mGnRH and cGnRH-II, has been addressed using phylogenetic approaches. Some authors (Okubo and Aida 2001) postulate that if only two GnRH forms occur in primitive teleosts such as eel and arowana (osteoglossiform), then the eel prepro-mGnRH and the arowana prepro-sGnRH should share high homology because they would be orthologues. In contrast, as they share low homology meaning they would rather be paralogues, the authors suggest that a gene duplication giving rise to mGnRH and sGnRH occurred before the emergence of teleosts and that a third form of GnRH may still be found in the eel (Okubo and Aida 2001). Other authors (O’Neill et al. 1998)
postulate that there was a substitution/replacement in the mGnRH gene after the eels evolved resulting in the sGnRH gene, based on the presence of sGnRH in four members of osteoglossiforms (*Osteoglossum bicirrhosum*, *Xenomystus nigri*, *Gnathorhynchus petersii*, *Chitala chitala*).

GnRH peptides bind to protein G-coupled receptors (GnRH-R) composed of an extracellular N-terminal region (30–40 amino acids), a large seven amino acid transmembrane domain (280–290 amino acids), and a short cytoplasmic C-terminal tail (30–50 amino acids). Within the teleost lineage, two main types of GnRH-R (termed type I and type II) could exist, each of which may include two or three subtypes (Lehmonnier et al. 2004). While the two types of GnRH-R in mammals have distinct selectivity, all the teleost types of GnRH-R have a higher affinity for eGnRH-II, followed by sGnRH and a third endogenous GnRH form (Lehmonnier et al. 2004). The two types of GnRH receptors have been identified in the Japanese eel (Okubo et al. 2000), and also in goldfish (Ilting et al. 1999; Peter et al. 2003), African catfish (Tensen et al. 1997; Bogerd et al. 2002) and pufferfish (Yumoto et al. 2001). The situation may be still more complex, as recently, five different subtypes of GnRH-R were detected in masu salmon (Jodo et al. 2003), in the spotted green pufferfish *Tetraodon nigroviridis* (Ikemoto and Park 2005) and in European sea bass (Moncaut et al. 2005), while three forms have been reported in medaka (Okubo et al. 2001, 2003) and tilapia (Soga et al. 2005). In other species, up to now, only one type of GnRH-R was reported (rainbow trout: type II; Madigou et al. 2000; striped bass: type I; Alok et al. 2000). Further studies aiming at cloning and characterizing GnRH-R types and eventual subtypes in the European eel are clearly needed.

## 11.3 Gonadotropins

As in other vertebrates, the teleost pituitary secretes two gonadotropins (GTHs), follicle-stimulating hormone (FSH, formerly designated as GTH I in fish) and luteinizing hormone (LH, formerly GTH II in fish). GTHs are heterodimeric glycoproteins composed of a common α subunit and a hormone-specific β subunit. Our recent study in the eel using *in situ* hybridization demonstrated that LH and FSH were expressed by separate cells in the proximal pars distalis of the pituitary (Schmitz et al. 2005). In other teleost species, they are also produced in different cells in the pituitary (salmonids: Nozaki et al. 1990a; Naito et al. 1993; tuna: Kagawa et al. 1998; tilapia: Melamed et al. 1998; gilthead seabream: Garcia Ayala et al. 2003; halibut: Weltzien et al. 2004; zebrafish: So et al. 2005). This distinct cellular source for the two GTHs in teleosts may facilitate the distinct regulation of gonadotropin synthesis and secretion. In contrast, in mammals, it was demonstrated that FSH and LH were expressed by the same gonadotropic cells (Childs et al., 1986; Liu et al., 1988). LH and FSH β subunits as well as the common glycoprotein α subunit (Gpα) have been cloned in the European eel (Querat et al. 1990a, b; Schmitz et al. 2005) and in the Japanese eel (Nagae et al. 1996a). Measurement of their mRNA indicated that FSHβ increased at the early steps of the silverying
process, while LHβ increased strongly later in the silverying process (European eel: Aroua et al. 2005; Rousseau et al. this book).

Fish gonadotropins act via binding to two gonadal gonadotropin receptors, homologous to mammalian LH receptor (LH-R) and FSH receptor (FSH-R). In contrast to the situation in mammals, the interactions are not highly specific (for review: Bogerd et al. 2005). Indeed, the catfish FSH-R is highly responsive to both catfish LH and FSH (Bogerd et al. 2001; Vischer et al. 2003), while the LH-R is rather specific to LH (Vischer and Bogerd 2003). The same situation is observed in coho salmon (Miwa et al. 1994; Yan et al. 1992). However, studies in zebrafish (Kwok et al. 2005), amago salmon (Oba et al. 1999a, b) and seb gill (Rocha et al. 2007) showed that FSH-R was specific to FSH, while LH-R was activated by both LH and FSH. Two types of gonadotropin receptors, respectively homologous to other teleost LH-R and FSH-R have been recently cloned in the Japanese eel (Jeng et al. 2007). Measurement of their ovarian mRNA levels by absolute quantitative real time RT-PCR indicated that FSH-R expression was much higher (50-fold) than that of LH-R in the previtellogenic eel (Jeng et al. 2007). In immature male Atlantic salmon, FSH-R transcripts are also more abundant (8-fold) than LH-R ones (Maugars and Schmitz 2007). Maturation experiments in the eel indicated that human chorionic gonadotropin, a LH-like hormone, was unable to induce ovarian development, which can be triggered by fish pituitary extract. This suggests that eel FSH-R may have a strong specificity and does not recognize mammalian gonadotropin. Our future investigations will aim at cloning European eel gonadotropin receptors and further characterizing gonadotropin receptor selectivity in the eel (Dufour, Chang and coworkers in the frame of the Taiwan-France international cooperation).

11.4 Sex Steroids

As in other vertebrates, the androgen, testosterone (T) and the estrogen, estradiol (E2) are present in teleost fishes. In addition, 11-oxygenated androgens, especially 11-ketotestosterone (11-KT) are also detected (Borg 1994). In mature male salmon parr and mature anadromous males, 11-ketotestosterone was even found to be the predominant androgen in the plasma (Mayer et al. 1990). Compared with adult mammals, the brain of most teleost fishes is characterized by an extremely high capacity to aromatize androgens into estrogens, because of exceptionally high levels of aromatase and of high aromatase activity (Callard et al. 1978; Pasmanik and Callard 1985; for review: Pellegrini et al. 2005). Unique among vertebrates, teleost fishes possess in fact three estrogen receptor (ER) subtypes (ERalpha, ER beta1, ER beta2) (Atlantic croaker, Micropterus undulatus: Hawkins et al. 2000; zebrafish: Bardet et al. 2002; rainbow trout: Mene et al. 2002; goldfish: Choi and Habibi 2003; European sea bass: Halm et al. 2004; fathead minnow, Pimephales promelas: Filby and Tyler 2005; sea bream: Pinto et al. 2006). Two androgen receptors (AR alpha and AR beta) have been identified in the eel (Ikuchich et al. 1999) as in many teleosts (for review: Douard et al. 2004). Different studies showed the
interdependence between androgen or estrogen receptors and aromatase in the brain of teleosts (for review: Pellegrini et al. 2005), with similar temporal patterns.

Low levels of androgens (mainly testosterone and 11-KT) are detected in the plasma of male silver eels (European eel: Khan et al. 1987; Japanese eel: Miura et al. 1991). Androgen production by eel testis is greatly stimulated during experimental maturation induced by hCG (European eel: Khan et al. 1987; Japanese eel: Ohta and Tanaka 1997; Japanese eel: Miura et al. 1991). In the female eel, plasma levels of T, 11-KT and E2 significantly increase between the pre-vitellogenic (yellow) and early vitellogenic (silver) stages as shown in Anguilla anguilla, (Sbaihi et al. 2001; Aroua et al. 2005) as well as in other eel species (A. australis and dieffenbachii: Lokman et al. 1998; A. rostrata: Cottrill et al. 2001; A. japonica: Han et al. 2003). Further increase in androgens and in E2 levels are observed during experimental maturation induced by gonadotropic (fish pituitary extract) treatments (Leloup-Hatey et al. 1988; Peyron et al. 1997). The similarity in plasma levels of androgens and estradiol is a remarkable feature in the female eel, likely related to androgen-specific regulations, as discussed in Chapter 12. Our recent study in Japanese eel showed that eel brain aromatase has a low activity compared to enzymatic activity in other teleosts (Jeng et al. 2005). This allows, in the eel, androgen-specific actions to be exerted, not only by non-aromatizable androgens such as 11-KT but also by aromatizable androgens, such as testosterone. Accordingly, testosterone-specific and estradiol-specific actions were found in the eel (see below). For the moment, no data are available concerning which androgen and/or estrogen receptor(s) is involved in the sex steroid feedbacks observed in the eel, on brain GnRHs and pituitary gonadotropins. However, concerning androgen receptors, in the Japanese eel only AR alpha is expressed in the hypothalamus (brain) (Ikeuchi et al. 1999).

11.5 Effects of Sexual Maturation on Eel Endogenous Brain-Pituitary Gonadotropic Axis

11.5.1 Effects on Gonadolibersins

As we mentioned before, silver eels remain blocked at a prepubertal stage as long as migration towards the Sargasso Sea is prevented, and to date, only experimental treatments of silver eels have led to the observation of sexually mature animals (male European eel: Fontaine 1936; female European eel: Fontaine et al. 1964). Based on these pioneer experiments in the European eel, similar treatments (hCG in males and fish pituitary extract in females) have been since currently employed to induce sexual maturation (gametogenesis and steroidogenesis) in various eel species (Anguilla japonica: Yamauchi et al. 1976; Ohta et al. 1997; Anguilla rostrata: Edel 1975; Sorensen and Winn 1984; Anguilla dieffenbachii: Todd 1979; Lokman and Young 2000). In the female, long-term treatment with carp pituitary extract stimulated ovarian vitellogenesis, leading to a gradual increase in gonado-somatic
index, which reached up to 30–40% after several months, an index much higher than in control eels (1.5–2%) (Fontaine et al. 1964; Dufour et al. 1989, 1993; Schmitz et al. 2005; Durif et al. 2005).

Our first studies, employing antibodies recognizing all forms of GnRH, indicated a positive effect of experimental sexual maturation on total GnRH level in the brain of female or male silver eels (males treated with hCG and females with estradiol: Dufour et al. 1985; females treated with pituitary extract: Dufour et al. 1989). This effect was even more marked in the pituitary, reflecting the accumulation of GnRH in the axonal endings, which are directly innervating the adenohypophysis in the eel as in other teleosts. These data were confirmed by immunocytochemical observation (Kah et al. 1989), which indicated a strong accumulation of GnRH peptide in the pituitary and, in particular, in the axonal endings of the hypophysiotropic neurons. Moreover, castration was able to abolish the increase in brain and pituitary GnRH content, which indicates that gonadal hormones are responsible for this positive effect (Dufour et al. 1989). Later on, using specific RIAs for each native form of GnRH in the eel, we could perform more specific analyses of the effect of experimental maturation on mGnRH and cGnRH-II. We were able to demonstrate an opposite regulation of the two forms with an increase in mGnRH levels in the brain and pituitary, whereas a decrease in cGnRH-II levels in the brain was found, cGnRH-II levels being not detectable by RIA in the pituitary (Dufour et al. 1993). This opposite regulation suggests that mGnRH and cGnRH-II play drastically different roles during eel sexual maturation, and that mGnRH would play a major role in the neuroendocrine control of pituitary gonadotropins.

A differential regulation of the two GnRH forms was also observed in the goldfish and the salmon, with an increase in sGnRH but not cGnRH-II in the anterior brain and in the pituitary during natural sexual maturation (Amano et al. 1992; Rosenblum et al. 1994). In (masu) salmon brain, sGnRH genes are activated long before sexual maturation (Ando et al. 2001). In the striped bass, the levels of the two most abundant forms in the pituitary, sbGnRH and cGnRH-II, increased during the autumn and peaked prior to (for cGnRH-II) and during (for sbGnRH) the natural breeding season in March to May (Holland et al. 2001).

In sea bass and striped bass, pituitary GnRH-R gene expression increases according to maturation (Alok et al. 2000). Similarly, in masu salmon, the different GnRH-R genes were shown to vary with the season and after a GnRH analog treatment (Jodo et al. 2005). Even though we may also expect an increase in pituitary GnRH-R during eel induced maturation, direct data on the regulation of eel GnRH-R are missing.

### 11.5.2 Effects on Gonadotropins

In the European eel, our early studies, using heterologous radioimmunoassay for carp LH β subunit, showed a large increase in pituitary LH content in artificially matured eels, namely in females treated with carp pituitary extract or in males treated with human chorionic gonadotropin (Dufour 1985). The effect of carp pituitary extract on pituitary LH content was prevented by ovariectomy (Dufour