# Energetic requirements and environmental constraints of reproductive migration and maturation of European silver eel (Anguilla anguilla L.)

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"Wetenschap is de titanische poging van het menselijk intellect
zich uit zijn kosmische isolement te verlossen door te begrijpen"
*Hermans*

"The works of nature are immeasurably superior to those of arts"
*Darwin*

"One can only understand the essence of things
when one knows their origin and development"
*Heracleitos*

"Nothing in biology makes sense,
except in the light of evolution"
*Dobzhansky*

"Altogether the whole story of the eel and its spawning
has come to read almost like a romance,
wherein reality has far exceeded the dreams of phantasy"
*Schmidt*

"After the riddle of the eel's gonads,
the exploration of the human psyche and the identification of the castration complex
must have been seemed comparatively straightforward"
*Fort about Freud*

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## The eel *Anguilla anguilla*

*Why eel? Commercial and scientific interests*

The European eel *Anguilla anguilla* is one of the most widely spread fish species in the European brackish and fresh water habitats. Eels are commonly found in basically all habitats from estuaries and rivers to lakes and canals, in the smallest ditches and even on land sometimes. The chance of an encounter with this species is therefore high. However, for by far most people such an experience would be considered as negative. Its snake-like appearance triggers a brain reflex for fear like with tails of rats and mice. Their unpredictable crawling movements, dark colour and slimy skin are characteristics that provide them with a low affection factor. This is reinforced by the untrue idea that eels are scavengers and the vultures of fresh water. A positive association for most people is its culinary quality. Eel has a great tradition in the European kitchens, recently well described by Schweid (2002). This is indicated by the fact that searching for the term eel on the internet mainly results in hits of recepies. In the Northern countries, large yellow and silver eel are eaten and prepared mostly by smoking. Catching and smoking of silver eels has a 6000 year old history since the Stone Age. It provided our ancestors yearly with high fatty food reserves in periods of scarcity between October and December. In the Iberic peninsula especially glass eel is eaten as a nowadays exclusive tapa at Christmas or as a surimi variant (Fig. 1) since prices have risen skyhigh (>1000 euros per kg). Eel is the only species that, when caught by sportfishermen, is almost never released and taken for cooking (Palstra, 2005).


**Figure 1** Since glass eel influx has collapsed and prices have risen skyhigh, much cheaper artificial Surimi glass eels are produced in countries where large scale consumption exists like in Spain.

Because of its culinary quality, eel is commercially interesting and a major target for fisheries and, more recently, for aquaculture. Eel fishery is the most widespread European fishery employing at least 25,000 people with a yearly turnover of 800 million euros (Dekker, 2004). The largest glass eel fisheries are found in France (Loire, Seine and Gironde; Fig. 2), Spain (Oria, Nalon and Minho) and England (Severn). Yield estimations that have been made are of an order of magnitude of 857 ton for the 80s and 500 tons for the 90s which are however for sure underestimations (Dekker, 2004). Only 20% of the yields are used for consumption. Another 20% is used for restocking. The remaining 60% is

used for aquaculture of which 50% is exported to Asia (Dekker, 2004). Aquaculture is still completely depending on fisheries since artificial reproduction is not successful yet. Also estimations of yellow and silver eel fishing yields are far from complete. The largest fisheries are found in The Netherlands, Denmark, Sweden and Ireland. Estimations show a decrease of yearly averages of 47,000 tons in the 60s down to 22,000 tons in 2004 (Dekker, 2004). Eel fishery created a rich cultural history in many villages along lake- and riversides. As for The Netherlands, the IJsselmeer fishery is the largest in Europe (Dekker, 2004) and the village of Volendam is famous for its eel folklore. Silver eel fishery is seasonly undertaken especially in the Haringvliet, Lake Grevelingen (Fig. 3) and the Oosterschelde.

**Figure 2** Glass eel influx at the sluices at Den Oever (photo Deelder, taken from Dekker, 2004).

Eel aquaculture follows the general trend in the fish industry and is gradually replacing fisheries. Eel aquaculture in Europe nowadays produces yearly 10,000 tons (Dekker, 2004) and thus accounts for a third of the total production. In Asia, production is 180,000 tons of all eel species together (Dekker, 2004). This mass production is illustrated by the fact that a complete Chinese city with a 100,000 inhabitants totally depends on the local eel hatchery producing 25,000 tons per year (Elie, pers. comment).

Over the past 25 years the population of European eel has been declining to such degree that major concerns have been raised for its long-term well being. Adult stocks have started to dwindle in the 40s in major areas of the continent, while recruitment (glass eel arrivals) has collapsed since the early 80s. The influx of glass eels has even declined with 99% (Fig. 4; Anonymous, 2003). There is no sign of recovery and the phenomenon seems to occur over the natural range of the European eel (*Anguilla anguilla*). A parallel development is observed in the closely related American eel (*A. rostrata* ; Castonguay et al., 1994*)* and Japanese eel (*A. japonica* ; Fig. 4). Concerns about the conservation of European eel has been growing during the last decade and the need for conservation and management measures has been clearly identified by scientists, managers, and even by the public at large. These have been expressed in the Quebec declaration of 2003 published in Nature (Clarke, 2003), Science (Stone, 2003), National Geographic (Owen, 2003), New Scientist (McKenzie, 2003), ICES Newsletter (Dekker, 2003) and Fisheries (anonymous, 2003). From the Commission to the Council and the European Parliament it was communicated (2003) that for the development of a community action plan for the management of European eel, it would be necessary to improve scientific research. All this did, however, not yet lead to measures for management and conservation and is still under debate. Voices are raised for temporal prohibition of all eel fisheries while preparing a management plan. Eel biology is dominated by many questions and only few answers. The story is therefore far from complete. Knowledge on its continental phase is provided for but from the moment they leave the continent until the moment glass eels swim upstream the rivers again, our knowledge suffers an almost total lack. The question where they are going is apparantly the same as the question where they come from.

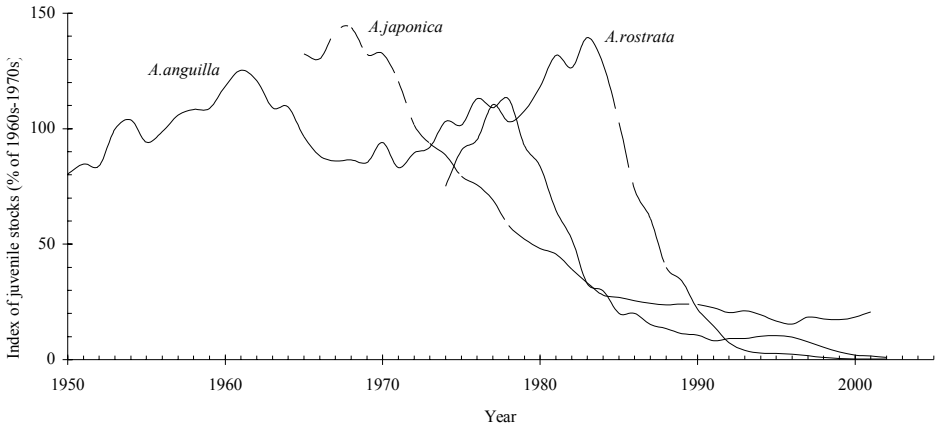


**Figure 4** Time trends in juvenile abundance of the major eel stocks of the world (taken from anonymous, 2003).

The glass eels start their feeding stage and are then refered to as yellow eels. At a certain moment, yellow eels start metamorphosis into silver eels and start migration. Still in

a prepubertal condition they leave to the spawning grounds and they never return. These spawning grounds might well be located in the Sargasso Sea (Schmidt, 1923). Eels are diadromous, migrating from salt water to fresh and back again as silver eels. This requires an extensive adaptive capacity. When they silver, they cease feeding. Purely on their fat reserves they are supposed to migrate 5,500-km to the Sargasso at depths of 500 to 1500 m and thus at high pressure. Only during this trip, which is supposed to last for 5 to 6 months, eels fully mature. This suggests a fine-tuning mechanism between migration and maturation and triggering of maturation by any of the experienced conditions. The lack of knowledge of natural triggers for eel maturation is probably one of the major reason why successfull artificial reproduction is still not possible.
Thus, eel biology is scientifically hot in both fundamental and applied aspects. It involves some of the most challenging biological challenges. Historically, eel challenged some of the greatest names: Aristotle, van Leeuwenhoek and even Freud.

### *Taxonomy and evolution of* Anguilla anguilla

The European eel reaches a maximum length of 133 cm, registered for an eel caught in Lake IJssel (The Netherlands; Dekker, 2004). Maximum weight is registered at 6,599 g. The oldest registered eel was 85 years old. European eel is a temporate eel inhabiting the European brackish and fresh water habitats (74°N - 25°N, 26°W - 45°E) between 4 to 20 °C. Their distribution is at the Atlantic coast from Scandinavia to Morocco and rivers of the North Atlantic, Baltic and Mediterranean countries (Fig. 5; all data from Deelder, 1984).
European eel *Anguilla anguilla* is one out of 15 species of freshwater eels of genus *Anguilla* (reviewed by Watanabe, 2003). Most well-known are the temperate species: the Atlantic species European eel (*A. anguilla*) and American eel (*A. rostrata*), Japanese eel (*A. japonica*), New-Zealand eel (*A. dieffenbachii*) and Australian eel (*A. australis*). Characteristics for all eels are their catadromous life history strategy, spending most of their lives in estuarine or inland waters in generalist feeding habitats, their long spawning migration and a semelparous spawn (Bertin, 1957; reviews Watanabe, 2003; Avise, 2003; Tesch, 2003), and certain spawning ground characteristics which determine its distribution (Fig. 6). The genus *Anguilla* belongs to the family of the *Anguillidae* and the order of the *Anguilliformes*. Also Muraenoidei and Congridae (Tesch, 2003) belong to this order which is specified by swimming in the anguilliform mode, typical for highly flexible fishes capable of bending more than half a sinusoidal wavelength. *Anguilliformes*, together with tarpons, ladyfish and bonefish, belong to the clade of the Elopomorpha that appeared in the early Cretaceous (Pough et al., 1996). Elopomorpha share some unique features. They share long generation times, breeding deep in the ocean and the unique character of specialized leptocephalus larvae that spend a long time at the ocean surface and are widely dispersed by currents. Elopomorpha are classified to the infraclass Teleostei (known for their advanced feeding and locomotor specialisations), the subclass Actinopterygii (ray-finned fishes) and the class Osteichthyes (bony fishes).
The origin of anguillid species was assumed to be somewhere in the Indo-Pacific region (Ege, 1939; Eckman, 1953), and this has not been disputed by recent (molecular) studies

(reviewed by Aoyama, 2003). Aoyama & Tsukamoto (1997) suggested that the ancient Tethys Sea, which separated Laurasia (North American and Eurasian continents) from Gondwana (Africa, South America and India), was the most likely dispersal route for the Atlantic species. Thus, the Atlantic population must have split off from the Indo-Pacific congeners before the closure of the Tethys Sea (approximately 30 million years). Based on this, the origin of anguillid eels was dated to approximately 50 to 60 million years. Fossil findings suggest that the family Anguillidae would have already appeared at least in the Tertiary, which seems to be congruent with the age estimation in the Tethys Corridor hypothesis (Aoyama et al., 2001).

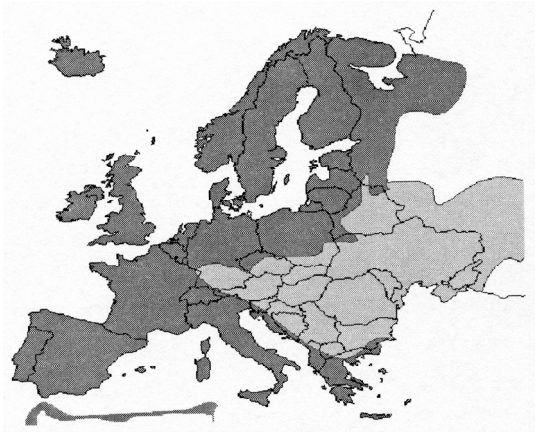

**Figure 5** The continental distribution of *Anguilla anguilla*. The dark grey area is the natural distribution area; light grey represents stocked populations (adapted from Lelek, 1987 after Maes, 2005)

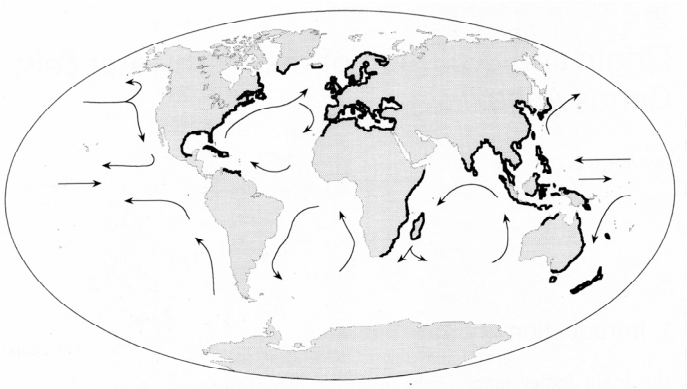

**Figure 6** Geographic distribution of the genus *Anguilla* (areas covered by thick lines). The basic patterns of current flow are indicated by arrows (taken from Aoyama, 2003).

CHAPTER 1

## The life cycle and world-wide collapse of populations

*Schmidt and the discovery of the Sargasso spawning grounds*

The mystery of the origin of eel has puzzled investigators throughout the ages. Eel was supposed to be spontaneously generated from mud, slime or drops of drew, to hybridize with snakes and to be bred by the sun's heat. Not until 1676 the first serious observations were reported by Redi (reviewed by Fort, 2002) stating that '*each year, with the first August rains and by night when it is most dark and cloudy, the eels begin to descend from the lakes and rivers in compact groups, towards the sea. Here the female lays her eggs, from which, after a variable time depending on the rigours of the sea, hatch elvers or young eels which then ascend the fresh water by means of the estuaries. Their journey begins about the end of January or the beginning of February, and finishes generally about the end of April*.' Grassi (1897) thought that he solved the mystery of the eel's life cycle. He and Calandruccio observed in the aquarium that the numerous Leptocephali that they found in the Straits of Messina were larval stages of European eel. Grassi stated that '*the abysses of the (Mediterranean) sea are the spawning places of the Common eel.'* However, all Leptocephali in the Strait of Messina were fully grown and none of the found specimens had been in their earliest stages of infancy.

It was the Danish ICES biologist Johannes Schmidt (1912, 1922, 1925, 1935) who traced back leptocephali during numerous oceanic explorations between 1904 and 1922 from the Mediterranean and found them smaller approaching an area south-east of the Bermudas (Fig. 7). Here he found the thiniest larvae of all at five millimetres in mid April. Schmidt might have missed spawning just by days. Never did he find corpses of dead parental eels, nor eggs, nor did he observe spawning. With this, the spawning area was pinpointed the most accurate until now. Fifty years later, Jan Boëtius did four expeditions in the Sargasso and also did not find mature eels nor eggs. He undertook the task of reviewing Schmidt's work (e.g. Boëtius and Harding, 1985). It was concluded that in essence, Schmidt had been correct. The segregation of spawning grounds of *A. rostrata* and *A. anguilla* is however still under debate.

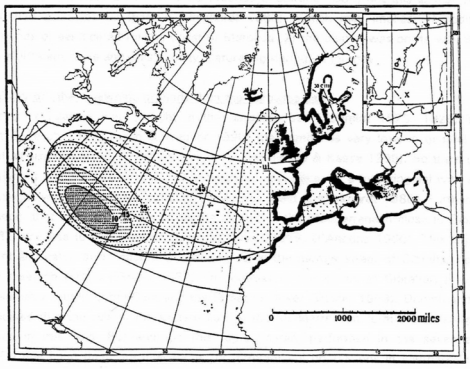


**Figure 7** Distribution patterns of eel larvae with the size of the larvae in mm (Source: Schmidt 1923).

There are indications that Schmidt's claim of complete homogeneity of the European eel population and a single unique spawning location, the panmixia theory, may be an overstatement (reviewed by van Ginneken & Maes, 2006). A dynamic discussion with arguments either supporting or rejecting panmixia follows the application of newly developed molecular markers. Studies by Lintas et al. (1998) and Bastrop et al., (2000) supported genetic homogeneity. Three following studies indicated a genetic mosaic consisting of several isolated groups (Daemen et al., 2001; Wirth & Bernatchez, 2001, Maes & Volckaert, 2002). The most recent study (Dannewitz et al., 2005) showed, however, that there was no stable spatial genetic structure when the number of sites and replicates in time was increased. So, at this moment we should still consider European eel as a homogenous population.

*The truly amazing life cycle of the European eel* Anguilla anguilla

With the discovery of the spawning grounds, a reasonable idea about the life cycle of the European eel could be obtained. A life cycle that is characterised by an oceanic and continental phase, two metamorphoses, a late environmental sex determination, an impressive long-distance migration and a strong reproductive inhibition (Fig. 8).

After hatching, Leptocephalus larvae are transported at shallow depths of 60-160 m. along the Gulf Stream and North-Atlantic Drift for a journey of seven to nine months back to the eastern Atlantic coast (Lecomte-Finiger, 1994; Desaunay & Guérault, 1997; Arai et al., 2000). Once arrived at the continental shelf, they display a first metamorphosis into small transparant glass eels (Tesch, 2003). They use tidal stream transport to migrate upstream the freshwater rivers (Edeline, 2005) at black moon. With high tide they surface and are transported inland, with low tide they look for shelter near the bottom (Tesch, 2003). Upon entering the fresh waters, glass eels will start pigmentation and can soon be considered as juvenile eels, already miniature forms of large yellow eels.

With occupation of a feeding habitat, the feeding stage starts and will last as long as 5 to 8 years for males and 8 up to 20 years for females (Tesch, 2003). Only when eels reach a size of about 30 cm, determination of the sex occurs. Sex may be genetically preprogrammed in eel but can apparently be overruled by environmental factors acting at regulatory steps of the control of gonadal sex determination. Eel can be feminised by oral administration of 17β-estradiol for four months during the juvenile stage as shown for Japanese eel (Tachiki et al., 1997). Sex determination seems density dependent and therefore determined by the environment. Bark et al. (2005) showed that on the west coast of Britain, high density river populations are dominated by rapidly maturing males. In contrast, east coast rivers support low population densities of predominantly larger long-lived females. This has consequences for timing of migration. Increasing density and competition might increase migration, with low densities suppressing the need to migrate (Knights, 1987). Thus, two life strategies seem to be reflected in which density dependent migration and sex determination allow optimal exploitation of available resources and maximal production of large highly fecund females. Similarly, the low productive Loire River in France is dominated by 96% large females while the high productive Fremur River is dominated by 70% males (Feunteun, 2005). Life history strategies are current topic of

investigations on Sr/Ca ratios by microchemistry on the otolithes (Daverat, 2005b; Tzeng et al., 2000; Tsukamoto et al., 1998). In general, these studies show only a facultative catadromy, since only a minor part seems to migrate far upstream. There is increasing evidence that leptocephali that metamorphose earlier are the ones that enter the fresh water (reviewed by Lucas & Baras, 2001). These eels are generally the large females growing slower, becoming older and having higher fat percentages (Wickstrom, 2005; Thibault, 2005). This justifies the conclusion that especially these females form an important component of the breeding stock when they eventually return to spawn (Knights et al., 1996), although Tsukamoto et al. (1998) suggested that eels from fresh water may not contribute significantly to reproduction. The smaller males have to swim a shorter distance than the large females minimising the sex-specific timing of the start of migration necessary to arrive at the same time.

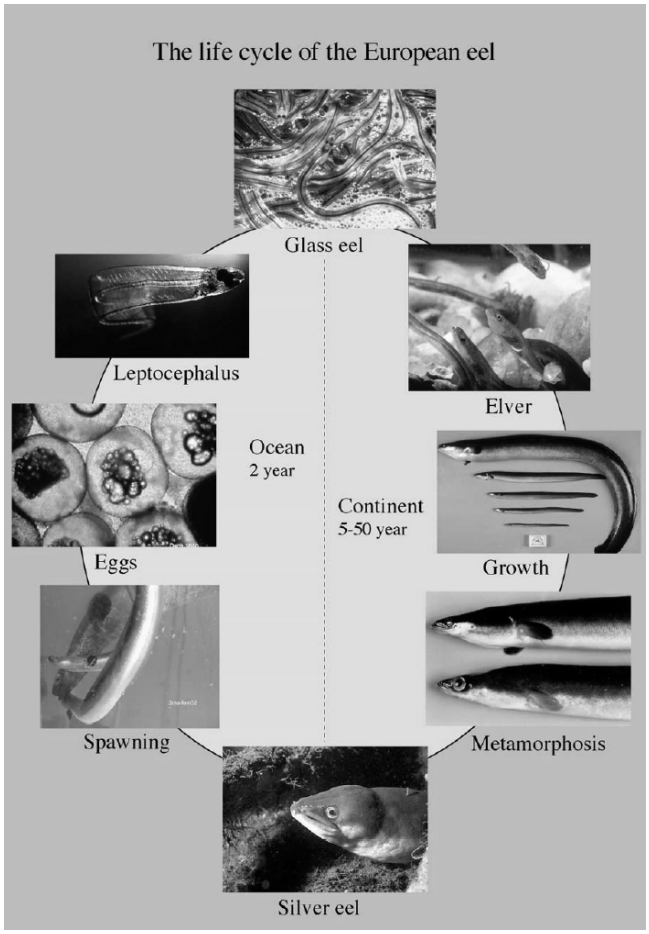


**Figure 8** Life cycle of the European eel (taken from van Ginneken, 2006).

*Silvering and freshwater migration*

European eels spend their feeding stage as immature yellow eels in the fresh and brackish European waters. It appears that at the end of each grow season in autumn certain eels cease feeding and metamorphose for a second time (silvering). Probably their fat content (Larsson et al., 1990; Svedäng & Wickström, 1997) plays a major role in the decision whether to start seaward migration or to regress silvering in following spring and start feeding again. A process which might be mediated through insulin and/or leptin (Huang, 1998; Dufour et al., 2000).

Drastic changes occur during silvering. Eels go through a number of morphological and physiological changes which will prepare them for their oceanic migration. The term silvering refers to the acquirement of a silvery shine. Externally, most apparent is the enlargement of the eyes which is used to discriminate between the yellow and silver phase in an index developed by Pankhurst (1982; Fig. 9). Also nostrils are enlarged and the lateral line is more visible (Dave et al 1974; Lewander et al 1974; Pankhurst 1983; Barni et al 1985). The head becomes more streamlined (Lokman et al., 2003) and the pectoral fins become longer (Durif et al., 2005) and change shape (Tesch, 2003). Since eels stop feeding (Fricke & Kaese 1995; Tesch 2003), their digestive tract regresses and the structure and metabolism of the liver changes too (Hara et al 1980). Internally, other changes occur like increase in gas deposition rates of the swimbladder (Kleckner, 1980a). Increased osmoregulation capacity is indicated by increased Na/K-ATPase activity and activation of filament chloride cells (Sasai et al., 1998). Changes occur in muscle composition e.g. an increase slow-cruising tonic fibres and in red muscle proportion (Ellerby et al., 2001) indicating increase in aerobic capacity and endurance. Changes also occur in blood chemistry and composition indicating higher aerobic capacity, for instance by increased haematocrit levels (reviewed by Lokman et al., 2003). Durif (et al., 2005) recently demonstrated that silvering and migration are closely related processes. On this basis, this author proposed an index on basis of length, weight, eye diameter and pectoral fin length which provides an estimate of the proportion of silver eels that are true migrants. This was needed since their abundance was overestimated as demonstrated by Svedäng & Wickström (1997) and Feunteun et al. (2000).

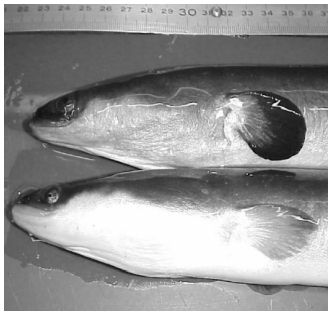

**Figure 9** Two large eels (80 cm) from Lake Grevelingen. The eel on top has larger eyes, a darker bronze skin and dark pectoral fins. This eel is silver according to the eye index from Pankhurst (1982) and migratory (stage 4 according to Durif et al., 2005), the eel below is also silver according to the eye index but still a pre-migrant (stage 3).

Light intensity is suggested to be a prevailing influence for migration. Eels are typically nocturnal except on cloudy days or at greater turbidity (review in Lucas & Baras, 2001). Silver eels migrate in autumn between sunset and midnight (Bruijs, 2005) at dark moon (Tesch, 2003; Todd, 1981; Vøllestad et al., 1986; reviewed by Jonsson, 1991), during high levels of water discharge and turbidity (Bruijs, 2005), at water temperatures between 9 to 12 ºC (Vøllestad et al., 1986). Eel migration ceases below 6 ºC (Tesch, 1972) and with the onset of frost (Frost, 1950).

It appears that silvering may well be a stepwise process which can be arrested at various stages as occurs for Atlantic salmon *Salmo salar* (Mills, 1989) and that silvering is more flexible than generally presumed (Svedäng & Wickström, 1997). For instance, in French Brittany, only about 20% of the silver eels actually did so (Feunteun et al., 2000). All together, the silvering process is a complex phenomenon and the actual sequence of events (intermediate phases), the link between external and internal modifications, as well as the duration of the silvering process remains still unknown.

During silvering, the first signs of an onset of maturity are indicated by an increase of gonadal mass reaching gonadal somatic index values between 1-2 (Tesch, 2003) and oocytes show an early development (reviewed for fish in general by Wallace & Selman, 1981 and Tyler & Sumpter, 1996, and for *A. japonica* by Adachi et al., 2003). At this stage, eels are still at a prepubertal stage and far from sexual maturity (Larsen & Dufour, 1993; Dufour, 1994; Dufour et al., 2003). The degree of inhibition is probably related to distance to the spawning grounds as illustrated by a negative correlation between migration distance and GSI at the start of oceanic migration of the various *Anguilla* species (Todd, 1981).

*Reproductive homing to the Sargasso, the characteristics of oceanic migration and fine-tuning between migration and maturation*

The reproductive migration to the Sargasso Sea is one of the most extreme homing (philopatry) examples of fish. Reproductive homing brings spawners back in conditions which are deemed favorable, since they permitted their own survival, and since they meet other mature spawners, thereby reducing efforts in search for mates (Wootton, 1990). Within an evolutionary perspective, traits will be selected that ultimately enhance reproductive success (Lucas & Baras, 2001). Traits involve the capacity for migration that relies on the integration of locomotor activity and associated energy provision, together with the ability to orientate in the direction of the overall migration goal (Lucas & Baras, 2001).

For piloting, orientation and navigation of homing fish in general multiple markers are succeedingly used. Markers may involve visual cues, celestial cues, currents, electric and magnetic fields, olfaction and gustation and others (Lucas & Baras, 2001). Olfactory stimuli for eels are in some cases detectable at concentrations of $10^{-16}$ M (Hara, 1993). During certain periods, especially during metamorphosis, elevated concentrations of thyroid hormones can be viewed as enhanced conditions for a ‘read’ (high responsiveness) or ‘write’ (imprinting) access to the long term memory (Lucas & Baras, 2001). Elevated levels in eels were found during both metamorphoses of leptocephali into glass eels (Ozaki et al., 2000) and of yellow into silver eels (Marchelidon et al., 1999). They were found to induce

increased locomotor activity in glass eels (Edeline, 2005). Cortisol might play a similar role (Lucas & Baras, 2001). The return of fish to previously occupied spawning places and associated orientation and navigation mechanisms intimately rely on the possibility of memorising characteristic features of the home area (Lucas & Baras, 2001).

The majority of the female eels leaves the continent in September-December and spawning is believed to occur primarily in March and April (McCleave et al., 1987; McCleave, 2003). Migration is generally assumed to last for 6 months at cruising swim speeds of around 0.4 m/s (or 0.5 BL/s for average female eels of 80 cm). A 30-year old history of tracking studies revealed speeds for migrating eels in the wild of 0.50-2.09 km/h (Tesch, 1974, 1978, 1989, 2003; Tesch et al., 1991; McCleave & Arnold, 1999; Jellyman & Tsukamoto, 2002) corresponding with 0.14-0.58 m/s (or 0.18-0.73 BL/s for an eel of 80 cm).

The exact route of migration is still largely unknown (reviewed by Tesch & Rohlf, 2003). Trackings of considerable numbers of eels in the North Sea and on the east Atlantic shelf have shown that eels swim uninterrupted in a compass direction geographically north and west. With decreasing latitude, directional preference turns over farther northward and attains in a NW swimming direction. The NW course must lead them to the continental slope where they start to swim in a SW direction. Eels that were released and tracked in the East Atlantic swam in a WSW direction. This kind of navigational ability could be based on magnetic sensing of the inclination or strength of the magnetic field. They use all depth zones, except for bottom layers, during all tidal phases. Swimming depth preferred by eels in the Baltic was temperature dependent Eels showed diel vertical migration during deep-sea migration and were ascending during dusk and descending during dawn. They migrated at depths between 200 and 600 m both in continental and deep-sea waters which probably persists as far as the spawning grounds. Fricke & Käse (1995) artificially induced maturation and released these eels at the supposed Sargasso spawning grounds. They preferred a depth of about 300 m which is in accordance with the depth range of newly hatched yolk sac larvae (Kleckner & McCleave, 1988).

Only three observations exist of migrating female silver eels in open ocean. Ernst (1975) reported a female that was caught near the Faroe Islands and had a GSI of 2.9. One female eel was caught near the Azores and had a GSI of 9.8 (Bast and Klinkhardt, 1988). Finally, Robins et al. (1979) phtotographed a migrating eel with swollen belly at the Bahamas at 2000-m depth (Robins *et al.* 1979). As for males, Grassi and Calandruccio (1896) caught a sexually mature eel in the Mediterranean. Also spawning places for Conger eels *Conger conger* are under debate (reviewed by Sbaihi et al., 2001). Suggested are the Sargasso Sea, the area between Gibraltar and the Azores at 3,000 to 4,000 m depth, and, also, the Mediterranean Sea. Cau & Manconi (1983) reported the capture of sexually mature male and female *C. conger* in 600 to 800 m deep water south-east of Sardinia.

When silver eels leave the continent they reflect a prepubertal condition at GSIs between 1-2. When they arrive at the spawning grounds about 6 months later they are sexually mature, ready to spawn at GSIs between 40-60 meaning that half of the body consists of more than a million eggs. A strong correlation between migration and maturation is supposed. Studies on the interaction between migration and maturation are scarce, which is surprising, since especially migrant fish are commercially interesting. Exercise has never been thoroughly investigated as stimulating factor for maturation of fish.

Only recently, van Ginneken et al. (submitted) found increased oocyte diameters in 3 year old hatchery eels after swimming for 5,500-km. Other triggers besides exercise that might be involved in induction of maturation might include area-specific odour, salinity and water temperature or the joining with the males (reviewed by Liley & Stacey, 1983 and Lam, 1983). Spawning ground specific triggering of the final stages of maturation has also been considered for other homing fishes like *Labeobarbus* (Palstra et al., 2004a).

*Potential actors of the worldwide collapse of eel populations: Quantity and quality of silver eel*

The European eel population is dangerously close to collapse. A great need exists for conservation and management measures. Essential data required for implementation in management models are lacking. Silver eel, as the contributing life stage to recruitment, is subjected to a number of anthroprogenic impacts. Silver eel can be considered as final product of the feeding stage and therefore as product of its feeding habitat. Habitat Evaluation Procedures (HEPs) have the objective to restore stock and habitats and take into account both quantity and quality (or suitability) of habitats (Klein-Breteler, 1996). Silver eel as product of its habitat can only contribute successfully to reproduction if quantity and quality are above a certain threshold. An essential question for management of stocks is then whether recruitment can simply be assessed as spawner quality*quantity. The anthroprogenic factors involved in quantity and quality of silver eel are the potential causes for eel's decline. Information regarding the quantity of silver eel is more provided that information about its quality. Quantitative factors involve:

1) Habitat degredation, meaning the physical loss of the habitat itself by land reclamation, swamp drainage or water course development (ICES, 2004; Klein-Breteler, 1996),
2) Restocking, of which it is assumed that these populations will not contribute to the spawning stock since they are not considered as being able to home to the Sargasso (Dekker, 2004). Westin (1998) provided information that silver eels, which originate as glass eels from the Atlantic coasts, have difficulty finding the outlets when leaving the Baltic. Occupation of food niches without contribution to reproduction is not an important reason of decline but is considered an anthropogenic forcing factor.
3) Overfishing, especially targeted fisheries on the vulnerable glass eel and silver eel aggregations (Dekker, 2004) with the latter suffering at least from 22% loss (Bruijs, 2005),
4) Migratory obstacles, like dams, pumping stations and hydro-electric plants causing maximally 16% mortality (Bruijs, 2005). Migrating silver eels in River Meuse have 30-40% chance to escape from fisheries and obstacles and to reach the sea (Bruijs, 2005).

Qualitative factors might involve:

5) Pollution, like accumulation of PCBs and endocrine related toxicants in fat of silver eel (Robinet & Feunteun, 2002), comparable with other fatty, migratory carnivorous fish like salmon (Hites et al., 2004). Migrating (silver) eels do not feed 'en route' and are totally dependent on their fat stores to fuel migration and gonad development. With fat consumption however, internal concentrations of lipophilic pollutants rise, thus increasing the risk for toxic effects. Eels often reside in contaminated sediments and

accumulate high levels of especially polychlorinated biphenyls (PCBs, van Leeuwen et al. 2002). These compounds have been shown to have adverse effects on fertility in fish (Stouthart et al. 1998) and amphibians (Gutleb et al. 1999) but also to disrupt mammalian oocyte maturation and follicle physiology in every species studied (Pocar et al. 2003). In a recent review, Robinet & Feunteun (2002) stated that ecotoxicological studies on the reproduction capacity of contaminated eels were not available.

6) Introduction of new diseases (*e.g*. the virus EVEX, Van Ginneken et al., 2004, 2005c) and parasite infections (*e.g.* swim-bladder parasite *Anguillicola crassus*, Haenen et al., 1994) by worldwide life eel transport (Van Ginneken et al., 2004). Both have been introduced recently in Europe from Asia. Eels infected with EVEX are not ill but they develop anaemia when stimulated to swim for several months. Also migration of eels infected with swim bladder parasite *Anguillicola crassus* is hypothesized to be impaired. Since both diseases have been introduced quit recently they can only act as accelerating causes of decline.

Not only anthropogenic and biotic factors are considered possible reasons for the decline. The connection between the recruitment decline in European eel and a decadal scale in the oceanic circulation (North Atlantic Oscillation – NAO) points towards those fluctuations as a possible cause of the decline (Castonquay et al., 1994; Knights, 2003). Correlation between the recruitment of the American eel and the NAO anomaly supports this model (McKenzie & Koster, 2004).

## Migration and maturation

### *Swim efficiency and the costs of transport*

Eel reproduction requires successful migration. High swim capacity and efficiency are necessary and presumed to be subjected to strong selection pressures that enhance evolutionary Darwinian fitness (Videler, 1993). Most likely the effective genitors contributing to the future generation are therefore characterised by an excellent swim fitness. Energy management is the key to successful migration, especially crucial since eels do not feed en route and thus completely rely on their reserves.

In contrast to human everyday life, fish live with neutral buoyancy in a dense medium. Basically, we still do not know the ultimate mechanism by which fish gain momentum by imparting force to water, in other words how they swim. In order to swim, they make use of structures like the muscles, body axis, fins, shape and skin. Fish in general can be viewed as two-gear animals being able to cruise for longer periods and being capable of sprinting occasionaly (Lucas & Baras, 2001). These two gears are respectively reflected in the two main types of muscle: 1) oxidative, slow-contracting red muscle provided with energy from aerobic metabolism for endurance and 2) fast-contracting white muscle provided with energy from anaerobic metabolism.

As stated, eels belong to the order of the *Anguilliformes*, swimming in the anguilliform mode, typical for highly flexible fishes capable of bending more than half a sinusoidal wavelength. Biomechanical efficiency of anguilliform swimming is considered

low (e.g. Lighthill, 1970; Videler, 1993) which is inconsistent with the situation in the field where silver eels migrate 5,500-km on their energy reserve stores (also Tytell & Lauder, 2004).

However, differences in swim performance between individual eels and between yellow and silver eels can be expected. Some of the changes that occur during silvering are associated in relation to swim efficiency. Ellerby et al. (2001) found that yellow-phase eels shifted to intermittent bursts of higher-frequency tailbeats at a lower swimming speed than silver eels. Morphologically, silver eel has acquired a more aquadynamic shape with the head becoming more streamlined in comparison with yellow eel (Lokman et al., 2003). The pectoral fins become longer (Durif et al., 2005) and shape changes (Tesch, 2003). These changes allow them to act as hydroplanes which provide lift. Neutral buoyancy is obtained by the high fat percentages around 20% (Svedang & Wickstrom, 1997) and by the swim-bladder that shows increased capacity (Kleckner, 1980a; Eggington, 1987; Kleckner, 1980b).

Eels exhibit a semelparous lifestyle meaning that individuals die after reproducing. The boundary between semelparous and iteroparous lifestyles seemingly lies within a 60-70% energy depletion range (Wootton, 1990). Iteroparous trouts spend 40-50% energy on spawning with 3-4% of gonadal energy (Jonsson, 2005). Semelparous salmons spend 70% on spawning with ranges of 75-82% in semelparous Pacific salmons (*Oncorhynchus* spp.) and 10% of gonadal energy. Brett (1965b) reported that salmon, migrating for about 1,000-km in 20 days, spends in total 96% of the fat supplies and 53% of the protein supplies. Like other fish species moving over considerable distances, eels accumulate significant amounts of energy in lipids within the muscles and around the digestive tract (reviewed by Lucas & Baras, 2001). The only calculations of required energetics for eel came from Boëtius & Boëtius (1980). They measured lipid and protein contents in ovaries of artificially matured silver eels (500-1500 g). They calculated on basis of two strip-ripe eels that 1.41 and 2.32 MJ was utilised for gonadal development. Furthermore they estimated, that after substraction of gonadal, lost and residual energy of the intial energy, 5.02 MJ of the energy was left-over for migration corresponding to 1.2 kJ/kg/km. Recently, large eels were subjected to long term swim trials in our swim-tunnel set-up (Fig. 10) in several studies aimed to estimate the ability and the energy costs to migrate and to compare efficiency with other fish species (Van Ginneken & Van den Thillart, 2000; Van den Thillart et al., 2004; Van Ginneken et al., 2005b). Results were quite revealing as it was found that eels swim 4 to 6 times more efficient than non eel-like fish and utilise only *c*. 60g fat per kg for migration, models describing propeller or muscle efficiency can not explain the high overall efficiency (Van Ginneken et al., 2005b).

The costs of migration are dependent on body size (Brett, 1965a; Brett & Glass, 1973) and the experienced conditions like swim speed (Brett, 1964; Tesch & Rohlf, 2003), water temperature (Brett, 1964), salinity (Kirschner, 1993, 1995) and depth (Sebert & Theron, 2001; Tesch, 2003).

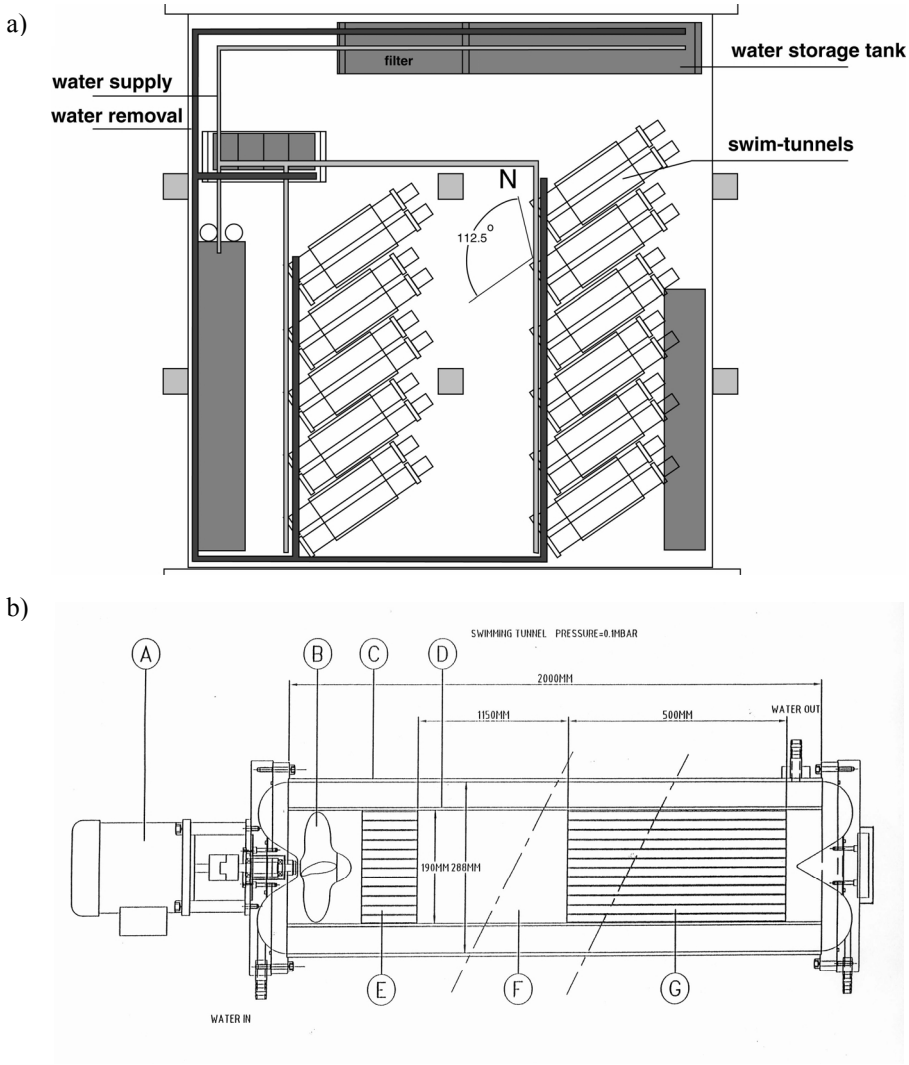


**Figure 10** a) Swim-tunnel set-up (7000 l) of 22 127-l Blazka-type swim-tunnels placed in a WNW direction in a climatised 100 $m^2$ room and b) Schematic drawing of a 2.0-m swim-tunnel. The tunnel consists of 2 concentric Perspex tubes of two-meter and two PVC endcaps. A: electromotor, B: propeller, C: Perspex outer swim-tunnel tube, D: Perspex inner swim-tunnel tube, E: PVC end-streamer, F: animal compartment, G: PVC front streamer. The propeller pushes water into the outer ring and sucks it out from the inner tube. The cross-section area of the inner tube and of the outer ring have the same surface area. This results in equal flow rates at both sides. The turbulent water is pushed through streamers that have internal diameters of about 10-mm (taken from van den Thillart et al., 2004).

CHAPTER 1

*Reproductive endocrinology and the dopaminergic inhibition of the pituitary*

In general fish endocrinology the process of maturation involves triggering of the hypothalamo-pituitary-gondal axis. Internal and external stimuli are perceived by the brain. In response, the hypothalamus secretes gonadotropin-releasing hormone (GnRH) that stimulates the release of gonadotropins (GtHs) from the pituitary. Two GtHs (GtH-I and GtH-II), structurally similar to respectively mammalian follicle-stimulating hormone (FSH) and luteinising hormone (LH), are secreted. In female fish, GtH-I is involved in vitellogenesis, while GtH-II plays a role in final oocyte maturation and ovulation (Swanson, 1991, Nagahama, 2000). GtH I induces the production of estrogen (17β-estradiol) by the granulosa cells (Kagawa, 1982, Nagahama, 2000), that stimulate the liver to produce and excrete vitellogenin (Nagahama, 1983). GtH-II induces the synthesis of 17α, hydroxy-4-pregnen-3-one by the theca cells of the follicle. This precursor is converted into 17α, 20β dihydroxy-4-pregnen-3-one (DHP) by the granulosa cells of the follicle (Nagahama and Yamashita, 1989). With this hormone maturation is triggered (release prophase 1 block) and ovulation is induced. With this stimulation the oocyte meiosis is reinitiated but again arrested at the metaphase in the second meiotic division, in which phase the oocyte is ovulated as a fertilisable egg.

For males, endocrinological regulation of gametogenesis is less clear. GTHs do not act directly but through steroid hormones that mediate various stages of spermatogenesis (Nagahama, 1994). Estrogen (17β-estradiol) is related to regulation of the renewal of spermatogonial stem cells through Sertoli cells in Japanese eel (Miura et al., 1999). Gonadotropin secretion induces an increase in 11-ketotestosterone, testosterone and DHP (Nagahama, 1987). 11-Ketotestosterone is synthesized in the testis inducing spermatogenesis from the proliferation of spermatogonia to spermiogenesis (Miura et al., 1991). 11-Ketotestosterone is an androgen that also seems to play a role in female eels, most probably during silvering (Rohr et al., 2001). In both male and female hepatocytes, 11-ketotestosterone enhances 17β-estradiol-induced vitellogenin production (Asanuma et al., 2003). The entry of spermatogonia into meiosis is also considered to be regulated by 11-ketotestosterone (Miura et al., 2003). All three 11-ketotestosterone, testosterone and DHP are assumed to be involved in final sperm maturation and spermiation (Miura et al., 2003).

Female European silver eels which are about to leave to the Sargasso Sea only exhibit gonadal somatic index values between 1-2. At this stage, eels are still at a prepubertal stage and far from sexual maturity (Larsen & Dufour, 1993; Dufour, 1994; Dufour et al., 2003). Prepubertal blockage of eel is due to a deficient GnRH stimulation and a simultaneous dopaminergic inhibition of the pituitary gonadotropes GTH-I (FSH-like) and GTH-II (LH-like) by dopamine. This dual neuroendocrine control is extreme, but not specific for eels and occurs in various adult teleosts (Vidal et al., 2004). However, here dopamine only counteracts regulation of the last steps of gametogenesis. In eel, dopamine seems to play a role in earlier stages (Vidal et al., 2004).

Lokman et al. (2003) reviewed the hormonal control of silvering in field-based and experimental studies. Comparison of yellow and silver eels from the field showed increased serum thyroxine, 17β-estradiol, 11-ketotestosterone and pituitary GTH-II and a lowered

pituitary GH (also Durif et al., 2005) in silver eels. Rohr et al. (2001) reported numerous silvering-like changes resulting from 11-ketotestosterone implants in female *A. australis*.

*Oocyte development*

The ovaries of teleost fishes are generally paired structures attached to the body cavity (Nagahama, 1983). Most teleosts are oviparous and release yolky eggs into the external aquatic environment where they are fertilised. Three basic patterns of oocyte growth can be recognized (Marza, 1938): synchronous, group synchronous and asynchronous development. Synchronous ovaries are mainly found in semelparous teleosts, which only spawn once and then die; for example anadromous *Oncorhynchus* species, catadromous salmonids (Dye et al., 1986) and eels (Wallace and Selman, 1981). In asynchronous development oocytes of all developmental stages are present, without dominant populations. Ovulation can occur continuously during the breeding season with pools of oogonia available for recruitment (Wallace and Selman, 1981; Evans, 1997). In group synchronous development two or more distinct populations of oocytes are present at the same time (Wallace and Selman, 1981; Evans, 1993). Multiple ovulatory events can occur.

Proliferation of primordial germ cells in the ovaries give rise to a stem cell population of oogonia. These oogonia are found in cell nests where they keep dividing (Billard et al., 1982; Tyler & Sumpter, 1996). During oogenesis, oogonia transform into oocytes. With this, the oogonium enters meiosis and the chromosomes become arrested at the diplotene of the first meiotic prophase (Tokartz, 1978). Oogenesis is followed by a growth phase and a maturation phase. The growth phase can be divided into a primary and secondary growth phase. The primary growth phase consists of a chromatin nucleolar and perinucleolar stage. The secondary growth phase consists of a previttelogenic stage (lipid vescicle stage) when fats are incorporated and a vitellogenic stage when vitellogenin is incorporated in yolk globuli. After entering meiosis the oocyte is at the beginning of the primary growth phase in the chromatin nucleolar stage. The oocytes are still in the nests. During this phase intense RNA synthesis occurs. There is one centrally located nucleus and one nucleolus. Also there is the formation of the Balbiani body (Guraya, 1979) which is a complex of organelles such as: mitochondria, endoplasmatic reticulum and golgi elements. An acellular vitelline envelope develops around the oocyte, which is called the zona radiata or the chorion vitelline envelope. At the end of the primary growth phase oocytes migrate out of the nest regions and enter the follicular phase. Steroid-secreting granulosa and thecal cells increase in number and form a continuous layer around the oocyte. The oocytes then enter the perinucleolar stage or follicular phase. The nucleus increases in size and several nucleoli appear due to a amplification of ribosomal genes (Vlad, 1976). Next to the Balbiani body another body can be seen at the primary growth stage: an island of lightly staining cytoplasm (Wallace and Selman, 1981) with unknown components. The theca and granulosa cells are responsible for production of reproductive steroid hormones that regulate successive stages of reproduction. Furthermore, during the primary growth phase the oocyte can considerably grow in size. It is known that oocytes of the rainbow trout

(*Oncorhynchus mykiss*) can increase in volume to at least 1000 up to a 5000 fold (Nagahama, 1983).

During the lipid vescicle stage (or cortical alveolus stage) are cortical alveoli the first cytoplasmic structures within the oocyte and appear during the gonadotropin-depended growth phase (Konopacka, 1935). These structures contain a polysialo-glycoprotein and appear to be synthesised endogenously. Towards the end of this growth phase, the cortical alveoli almost entirely fill the oocyte cytoplasm. Lipids are incorporated in vescicles. During this stage they do not cover yet >50% of the cytoplasm and form a complete ring around the circumference of the developing oocyte (Couillard et al., 1997), clear stage 3 previttelogenic oocytes (Colombo et al., 1984).

The principal event responsible for the enormous growth of oocytes in many teleost is vitellogenesis (Wallace & Selman, 1978). Vitellogenesis is responsible for the synthesis and uptake of vitellogenin, egg yolk proteins, which provide nutrients for the developing embryo. Besides fast growth, the oocytes increase in transparancy. Both are considered as a result of fusion of yolk globuli (Wallace & Selman, 1981). Vitellogenesis in Japanese eels starts at an oocyte diameter of 250 µm (Adachi et al., 2003) and is indicated when >50% is covered by fat droplets in *A. rostrata* according to Cottril et al. (2001). Throughout vitellogenesis, there is a continuous interaction between the pituitary in the brain, follicle cells, liver and the eggs (Fig. 11). The pituitary produces gonadotropins (GtH I and II), which are released into the blood circulation. These gonadotropins stimulate the theca and granulosa cells to produce oestradiol-17β (estrogen), which in its turn stimulates the liver to produce vitellogenin (Bidwell, 2000). In salmonids, production of follicular estradiol is most likely regulated by GtH I (Swanson et al., 1991). Vitellogenin penetrates the follicular cell layer through intercellular channels between the granulosa cells and reaches the oocytes via pore channels in the zona radiata (Abraham et al., 1984). Vitellogenin is taken up by the oocyte through specific receptor-mediated endocytosis and is then further converted into smaller yolk proteins. Vitellogenesis is the longest phase of oocyte development and requires a lot of nutrient input (Tyler et al, 1996).

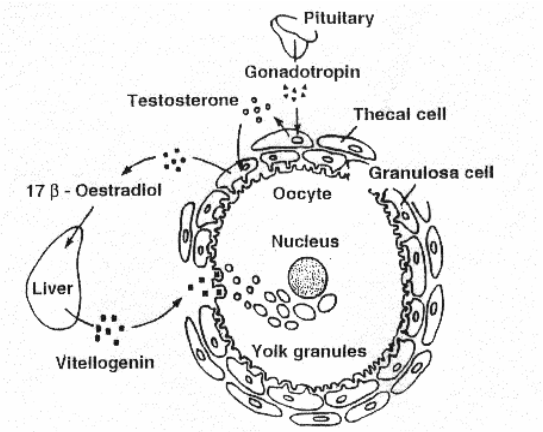


**Figure 11** Part of the hypothalamo-pituitary-gonadal axis showing hormonal interactions between pituitary, gonads and liver.

Oocyte maturation regards the period between prophase I-release and metaphase I-arrest (Evens, 2000). During the two meiotic divisions, the oocyte extrudes two polar bodies. During this process, the nucleus migrates from the centre of the oocyte to the periphery (Germinal Vescicle Migration GVM; Wallace and Selman, 1981). When meiosis resumes, the membrane of the nucleus breaks down (Germinal Vescicle Breakdown GVBD) and with the extrusion of a polar body the first meiotic division is completed (Huver, 1960). The second meiotic division starts immediately thereafter, but develops only up to the metaphase stage (Goswami and Sundararaj, 1971; Masui and Clarke, 1979). Hydration is important in species that produce buoyant eggs and is seen typically in marine teleosts. The acquisition of buoyancy in such species is an essential event in reproduction and effects both fertility and survival of spawned eggs (Carnevalli et al., 1999). The mechanism of hydration during maturation is not well understood, except that it does not seem to be caused by an osmotic adjustment to a change in ovarian fluid in which the oocyte-containing follicle is settled (Wallace and Selman, 1981). The osmotic gradient for water uptake during the hydration phase may be generated by secondary proteolysis of yolk proteins (Carnevali et al., 1991, 1992). The yolk also undergoes some sort of maturation as well as hydration and becomes less dense (Goswami and Sundararaj, 1971). Oil droplets, when present, coalesce to form one or more larger globules (Jalabert et al., 1973).

Recently, Pedersen (2003) described changes in diameter and appearance of oocytes during final maturation of European eel. Four oocyte stages were described (Pedersen, 2003): stage 1 (small, black non-transparent cells); stage 2 (larger eggs with a dark-grey cytoplasm containing numerous, small dark oil droplets); stage 3 (the greyish cytoplasm and the oil droplets are more transparent, oil droplets with increased diameter and decreased numbers); stage 4 (migratory nucleus with cytoplasm as well as oil droplets highly transparent). The different stages were, however, not described in more detail and were not used to describe the oocyte stage distribution during final maturation.

Ovulation refers to the expulsion of the mature (secondary) oocyte from its follicular envelope (Evens, 1997). The separation of microvillar processes between the follicle and the oocyte is followed by the rupture of the follicle cell layer. In catfish (Goswami and Sundararaj, 1971) and yellow perch (Goetz, 1979), maturation in vitro is often followed by ovulation, whereas in rainbow trout maturation and ovulation are distinct phases. The steroid responsible for stimulating maturation may also initiate a process to bring about detachment of oocyte from the follicle (Jalabert, 1976). Eventually, the ovulated eggs (ova) are released in the water and almost immediately fertilized by the male fish. A narrow time window exists for ovulation and fertilisation. Oocytes over ripen in several days and sperm is motile for about 30 to 60 s (Coward et al., 2002).

### *Artificially induced maturation and spawning*

Basically, eel's maturation from puberty to death can be largely considered as a black box (Dufour et al., 2003). The most advanced stage of maturation that we know from the field situation is the prepubertal silver eel leaving for the Sargasso. This knowledge is mainly acquired from artificially induced maturation and from few investigations on natural inducers and incidental observations from the field. The quest for successful artificial

reproduction has a long history, but is still open. Success is needed because aquaculture relies exclusively on the yearly influx of glass eels, which has declined by 99% since 1978 (anonymous, 2003). If successful, aquaculture could be provided with artificially bred stock while the natural populations have a chance to recover without fishing pressure on the yearly arriving glass eel stock.

The history of artificial reproduction of European eel started with Fontaine and co-workers at the National Museum of Natural History of Paris (reviewed by Dufour, 2003). Fontaine performed pioneer work on the induction of sexual maturation in eel. He discovered that injection of urine extract from pregnant women (known later to contain large amounts of human chorionic gonadotropin, hCG) induced full spermatogenesis in males (Fontaine, 1936). Injection of carp pituitary extract (CPE) induced ovarian development in females (Fontaine, 1964). More than 20 years ago Boëtius & Boëtius (1980) were able to fertilise eel eggs and Bezdenezhnykh et al. (1983) obtained eel larvae. Of the latter, however, very little evidence was provided and they died within a few days after hatching.

In Japan, hormonal treatments have been intensively applied to induce maturation in Japanese eels since the 1960s. In the 70s, Yamamoto and his colleagues succeeded in the production of larvae (Yamamoto & Yamauchi, 1974; Yamauchi et al., 1976). Ever since, the emphasis has been on the feeding of larvae (Tanaka, 2001) as well as on improvement of the maturation protocol. Since 1997, artificial reproduction of Japanese eel (*Anguilla japonica*) has become more successful with the application of 17, 20 β-dihydroxy-4-pregnen-3-one (DHP) for final maturation and ovulation resulting in fertility and hatching rates of 89.6 and 47.6% respectively (Ohta et al., 1996). Lokman & Young (2000) used Ohta's (et al., 1996) protocol on New Zealand freshwater eels (*A. dieffenbachii* and *A. australis*). They obtained larvae of *A. australis* and kept them alive for a few days. Tanaka et al. (2001) developed a successful larval slurry-type diet on basis of krill extract and freeze-dried shark egg power. Larvae have been raised on this diet for more than 200 days up to the glass eel stage (Fig. 12).

The Japanese researchers used the developed protocol to induce ovarian development also in European eels (Chiba et al, 1994). However, until now they have been unable to reproduce European eel with same success as for the Japanese eel, showing that maturation of European eel differs to a great extent. They were however recently able to create hybrid *A. anguilla***japonica* larvae that stayed alive for 30 days (Okamura, 2004). Recently, Pedersen (2003, 2004) applied variations of the same protocol on European eel and obtained a few larvae that stayed alive for 2 days. Those larvae showed, however, delayed hatching and abnormal morphology.

What can be concluded after 69 years of studies on artificial reproduction of eel is that the trial and error approach did not lead to the desired result. Results are still poor. Timing of ovulation, stripping of gametes and/or spawning is crucial but yet not established. Time of priming and induction of ovulation may, however, determine fertility.

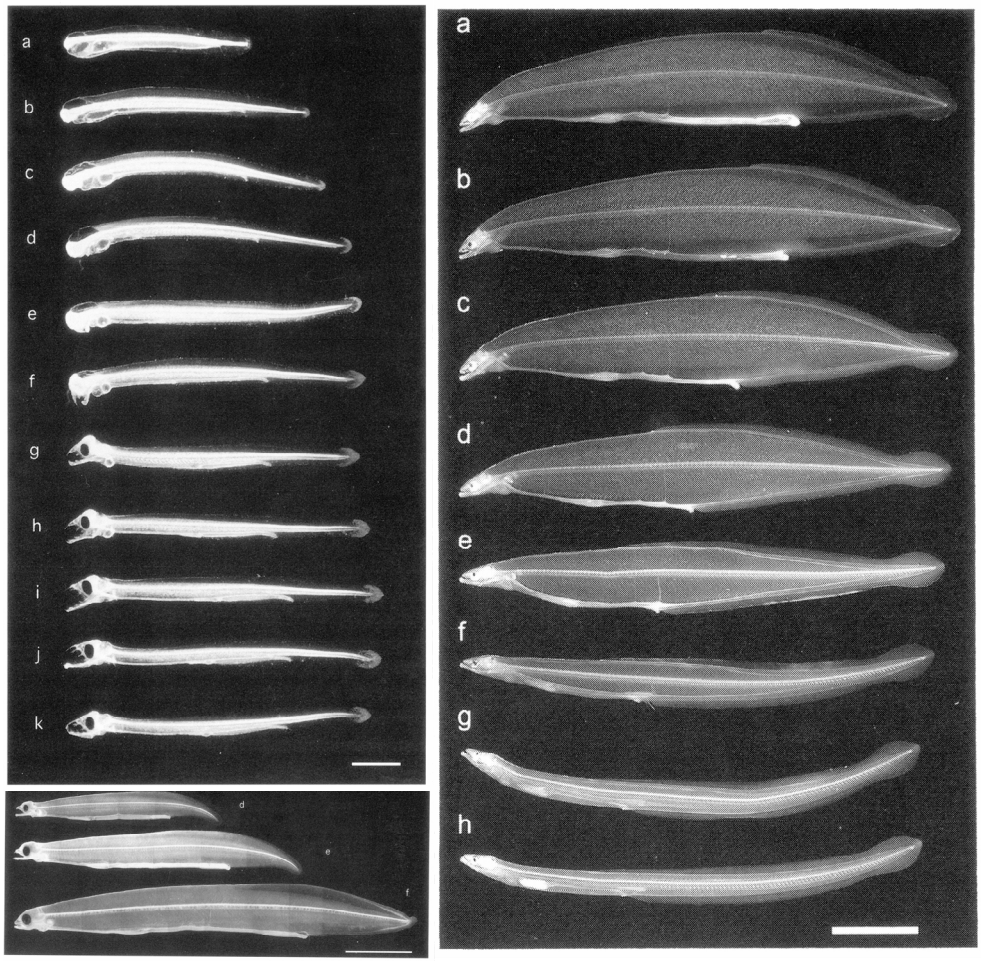


**Figure 12** Captive-bred *A. japonica* preleptocepahli, leptocephali and glass eels from hatching to 270 days after (taken from Tanaka, 2003).

*Requirements for reproductive migration and maturation: the objectives of this study*

The red line in this study involves the mysterious last phase in the life cycle of European eel. The period between the start of silvering and migration, coinciding with maturation from prepuberty until spawning. Most certainly the effective genitors are characterised by an excellent swim fitness. Highly efficient energy management is required, not only to fullfill migration, but also to provide the developing oocytes. When the silver eels leave they are still in a prepubertal condition, while after six months swimming they should be fully mature and ready to spawn. Information about migration, maturation and

interaction and fine-tuning between these two is lacking. Understanding of natural triggers for maturation, likely by swim exercise itself, could lead to more successful reproduction protocols. Information on these topics is required both for management of natural eel stocks and fisheries, as well as for successful eel aquaculture.

**Chapter 1** provides a general introduction followed by chapters 2 to 7 i.e. the experimental chapters. Chapter 1 provides general information about eel, it describes the amazing life cycle and the potential causes of eel's decline. Subsequently, the status of recent knowledge is described concerning aspects of eel migration and maturation introducing the objectives of this study.

Information on the swim efficiency of large migratory silver eels is limited. Only very recently, our group found that eels swim 4 to 6 times more efficient than non eel-like fish (van Ginneken et al., 2005b). Swim performance likely varies among silver eels within and between locations, mainly determined by the quality of the habitat in which they reside. Simulated migration trials would take too long to test differences between groups of different locations, and under different conditions. Therefore in **chapter 2**, our first objective was to develop a swim fitness test providing a fast impression of swim capacity and swim efficiency. This test could be applied to investigate the swim performance of silver eels from different locations.

Infection with swim-bladder parasite *Anguillicola crassus* is suggested as a cause of the collapse of eel populations. This nematode has been introduced 20 to 30 years ago from Asia and parasitised in short time various eel species in different geographical regions of the world. The effects are energy drain due to its sangivorous activities and mechanical injury of the swim-bladder wall by its migratory activity. These effects are hypothesized to impair the 5,500-km reproductive migration of European eel to its spawning grounds in the Sargasso Sea. In **chapter 3**, we have applied the swim fitness test to investigate the effects of infection on swim performance. We hypothesized that parasitic sanguivorous activities cause energy drain and reduce swim endurance. Furthermore, we hypothesized that the mechanical injury caused by migratory activity of the parasites impairs buoyancy control. Eels from Lake Balaton (Hungary) show high infection levels at the end of the summer. Eighty of these eels, varying in severity and developmental stage of infection, were subjected to the developed swim fitness test.

European eel is a primitive teleost with a semelparous life style and is one of the most extreme examples of reproductive homing. They migrate downstream and leave the European coasts as silver eels in a prepubertal condition to arrive 4 to 5 months later in a mature condition at the spawning grounds in the Sargasso. We consider it very likely that swim exercise triggers maturation during the 5,500-km migration as shown by results of van Ginneken et al. (unpublished). We hypothesize that swimming is involved in metamorphosis (silvering) and in release from reproductive inhibition and depressed lipid mobilisation. In **chapter 4**, we subjected 55 old (>13 years) eels from Lake Balaton (Hungary) to swimming for durations of 1, 2 and 6 weeks. Changes in morphometry and oocyte development were investigated to establish the silvering and maturation status.

Since silver eels seem to disappear in the ocean, it has not been possible yet to study the final stages of natural maturation. Attempts on artificial maturation and reproduction of European eel (*Anguilla anguilla*) have largely been unsuccessful. The final stages of oocyte maturation have not been described in detail yet. Such knowledge is

crucial for the time of priming and induction of ovulation which may determine fertility. In **chapter 5**, we artificially induced maturation of male and female European silver eel from Lake Grevelingen (the Netherlands) by hormonal injections. Oocyte development during final maturation was followed and cytological changes were studied and categorised with the purpose to improve timing of priming and induction of ovulation. Improvement of fertility was tested by stripping and mixing male and female gametes.

Ecotoxicological studies on the reproduction capacity of contaminated eels are not available. Until now, it was not possible to study the effects of contaminants on fertility and embryonic development since artificial reproduction has been unsuccessful. In the study of chapter 5 and a subsequent study, we were able to fertilise eggs and follow embryonic development. Large differences were observed with respect to development in fertilised egg batches. We hypothesised that this was caused by maternal dioxin-like contaminants deposited in the egg yolk. Therefore we measured in **chapter 6** the levels of dioxin-like compounds in muscle and gonad tissues from these eels and correlated their distribution to embryonic survival and development.

The energy budget of semelparous eels has to be a true example of biological efficiency. They cease feeding and metamorphose (silvering) preparing for their spawning migration to the Sargasso. Their stored energy, mainly as lipids in muscle and under the skin, should suffice for both migration and incorporation in the oocytes in order to reproduce successfully. Few attempts however were made to estimate the energetic costs. In **chapter 7** we subjected cultured eels and wild large silver eels to simulated migration and calculated cost of transport from oxygen consumption rates and the required amount of fat. Furthermore, we artificially matured eels from the same batch by hormonal injections to determine the amount of fat that was incorporated in the oocytes. Calculation of the sum of fats required for migration and maturation will represent the fat requirements for reproduction.

**Chapter 8** provides a summary of all results and the main conclusions.

CHAPTER 1

CHAPTER 2

A large silver eel swimming in one of the 22 swim-tunnels.

# Chapter 2

**Swim performance of European silver eels (*Anguilla anguilla*)**

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CHAPTER 2

ABSTRACT
The 5,500-km migration to the spawning grounds in the Sargasso is crucial for reproduction of European eel. Most certainly the effective genitors contributing to the future generation are therefore characterised by an excellent swim capacity and efficiency. Performance is likely to vary among silver eels within and between locations, mainly determined by trophic quality. In order to be able to compare the performance of farmed and wild eels from different locations and under different conditions, we developed a swim fitness test. Swim trials with 101 female eels (60-96 cm, 400 – 1500g) were performed in 22 Blazka-type swim-tunnels in a climatised room at 18ºC with running fresh or salt water. Speed and endurance swim trials started at 0.5 up to 1 meter per second (m/s) with increments of 0.1 m/s. Since both tests showed similar results, the single day speed test can be used to predict endurance. Eels reached maximum aerobic swim speeds of 0.81 up to 1.24 BL/s body-length per second (BL/s). At optimum swim speeds of 0.74-1.02 BL/s, the cost of transport (COT) values were 37-50 mg $O_2$/kg/km, which are very low in comparison to other fish species. Energy expenditure during exercise was 20% higher in SW than in FW. Wild silver eels showed lower performance than farmed silver eels. Overall, we can conclude that silver eels can be considered as cruising specialists. If silver eels cruise at their optimum swim speeds, they would travel in less than 3.5 months to the Sargasso instead of the generally assumed 6 months.

## INTRODUCTION

The 5,500-km migration to the spawning grounds in the Sargasso (Schmidt, 1923) is crucial for the semelparous reproduction of European eel. Swim capacity and swim efficiency are therefore primary necessities and presumed to be subjected to strong selection pressures that enhance evolutionary Darwinian fitness (Videler, 1993). Most certainly the effective genitors contributing to the future generation must therefore be characterised by an excellent swim fitness. A strong correlation between Darwinian fitness and swim fitness for silver eels is therefore to be expected.

The onset of migration is preceeded by and correlated to metamorphosis of ‘continental’ yellow eels into silver eels (‘silvering’), a physiological and morphological preparation for their oceanic journey (Tesch, 2003; Lokman et al., 2003; Durif et al., 2005). Because of the cessation of feeding, silver eels have to primarily rely on their fat stores for swimming and reproduction. Fat, protein and carbohydrate are metabolised in the same proportion, since body constitution does not change during migration (Van Ginneken et al., 2005b) and maturation (Palstra et al., 2006). Drastic changes occur during silvering. Most apparent is the enlargement of the eyes which is used to discriminate between the yellow and silver phase in an index developed by Pankhurst (1982). Recently, Durif (et al., 2005) developed a more detailed index in which silvering is correlated to migration. Silvering also involves aquadynamic adaptations. The head becomes more acute and streamlined (Lokman et al., 2003). The pectoral fins become longer (Tesch, 2003; Durif et al., 2005) and change shape (Tesch, 2003). These changes allow them to act as hydroplanes that provide lift. Neutral buoyancy is obtained by the high fat percentages up to 35% ((Svedäng and Wickström, 1997) and by the swim-bladder which also shows increased capacity

(Kleckner, 1980a; Eggington, 1987; Kleckner, 1980b) in order to migrate at depths between 200-600 m (reviewed by Tesch & Rohlf, 2003). Migration in the field is generally assumed to last for 6 months at cruising swim speeds of around 0.4 m/s or 0.5 BL/s for average female silver eels of 80 cm. A 30-year old history of tracking studies revealed speeds for migrating eels in the wild of 0.50-2.09 km/h (reviewed by Tesch, 2003; McCleave & Arnold, 1999; Jellyman & Tsukamoto, 2002) corresponding with 0.14-0.58 m/s or 0.18-0.73 BL/s for an eel of 80 cm. Female eels leave the continent in September-November and spawning is believed to occur primarily in March and April (McCleave, 2003). The much smaller male silver eels (40 cm) should leave earlier to arrive in time.

In the 60s and 70s intensive research has been performed on the fundamentals of salmonid, swimming. Jones and Randall (1978) reviewed the pioneer work of especially Brett (1964, 1965ab) and Glass (1973), providing the parameters for quantification of sustained exercise. When measuring sustained exercise, it is generally assumed that respiratory and circulatory adjustments are adequate to meet increased energy demands aerobically. It is assumed that the anaerobic energy contributions are negligible and that there are no changes in the mode of propulsion. An exponential relation exists between oxygen uptake and swim speed (*U*) like Brett (1964) found in sockeye salmon (*Oncorhynchus nerka*) and which depends on water temperature (Brett, 1964) and body size (Brett, 1965; Brett & Glass, 1973). The maximum oxygen uptake is achieved just before fatigue in an incremental velocity test. Since the drag on fish increases in proportion to $U^2$, so does the cost of transport (COT; also Fry, 1971). Since there is an exponential relationship between oxygen uptake and swim speed, an U-shaped relationship exists between COT and swim speed. The optimum swim speed is defined by the situation when COT is minimal. Only recently, such experiments have been continued, again mainly with salmonids (Lee et al., 2003ab).

Experimental data available on swim performance of *anguillids* is limited and concerns only small eels either juveniles < 15 cm (reviewed by Langdon & Collins, 2000) or intermediate sized, mostly yellow phase, eels (Schmidt-Nielsen, 1972; Webb, 1975; Van Ginneken, 2002). However, the silver phase is characterised by eel's impressive swim performance. Recently, we subjected large female eels to long term swim trials in several studies aimed to estimate the ability and the energy costs to migrate and to compare efficiency with other fish species (Van Ginneken & Van den Thillart, 2000; Van den Thillart et al., 2004; Van Ginneken et al., 2005b). Results were quite revealing as we found that eels swim 4 to 6 times more efficient than non eel-like fish and utilise only *c*. 60g fat per kg for migration. High efficiency might be expected for migrating silver eels in the field. However, biomechanical efficiency of anguilliform swimming is considered low (e.g. Lighthill, 1970; Videler, 1993) which is inconsistent with our findings (Van Ginneken et al., 2005b).

Swim performance is likely to vary among silver eels. Simulated migration trials would take too long to test differences between groups of different locations, and under different conditions. Our first objective is therefore to construct a swim fitness test providing a fast impression of swim capacity and swim efficiency. For this purpose, it is necessary to establish the relation between speed and endurance performance. This test can then be applied to investigate the swim efficiency of silver eels and the constraints. Constraints concern 1) size, 2) salinity, and 3) habitat. A larger eel is expected to perform

relatively worse but absolutely better than a smaller eel since performance is proportional to a fractional power of body length. Higher energy expenditure in SW is expected because of additional costs for osmoregulation (Kirschner, 1993, 1995). A better performance of wild eels is expected since they are less constrained than farmed eels in fat content and condition factor.

## MATERIALS & METHODS

*Experimental eels*

Eels were obtained from three locations:

1) Farmed eels were obtained from a commercial eel farm (Royaal BV, Helmond, The Netherlands) in October 2001 and September 2004. The October 2001 batches were acclimated to SW during a 2-week period and used in SW experiments in November and December 2001. The September 2004 batch was used in FW experiments within a week after arrival.
2) Wild migratory eels were caught during their seaward migration in a SW-habitat in the brackish Lake Grevelingen (The Netherlands). They were caught in November 2001 at the North Sea sluice at 32 ppt (Bout, Bruinisse, The Netherlands). They were used in experiments in January 2002.
3) Wild migratory eels from a FW-habitat in River Loire (France) were caught in November 2003 by local fishermen at Saint-Florent le Vieil between Angers and Nantes. They were used in experiments in December 2003.

*Swim-tunnel set-up and oxygen consumption*

A set of 22 Blazka-type 127-L swimtunnels (Blazka, 1960; Smith & Newcomb, 1970) as described by Van den Thillart et al. (2004) were used for the swim trials. The swim tunnels were placed in the direction of the Sargasso Sea (WNW) in a climatized room of about 100-$m^2$. The total water content of about 7000-L was recirculated continuously over a bio-filter. The illumination in the climatized room was switched to 670-nm light (bandwidth 20-nm). Based on eye pigment changes during silvering, it was assumed that this far-red light is invisible for eels (Pankhurst & Lythgoe 1983). Indeed the eels did not respond to movement of the experimentor during red light illumination. The oxygen level in the tunnel was measured continuously by an oxygen electrode (Mettler Toledo). The oxygen level in the tunnels was controlled as described before (Van den Thillart et al., 2004). If oxygen levels came below 75% air saturation, rinsing occurred automatically raising it up to 85%. The oxygen consumption rate was calculated from the oxygen decline after automatic closure of the water-inlet by a magnetic valve. From the decline of the $O_2$-concentration, the oxygen consumption rate was calculated following the formula 1 (Table 1).

*Pre swimming measurements*

Before introduction into the swim tunnels, eels were anaesthetized (MS222 250 ppm, benzocain 80 ppm or oil of cloves; 1:10 dissolved in 100% ethanol with 1-1.5 ml/l water) and tagged with small passive transponders for individual identification (TROVAN, EID Aalten BV, Aalten, The Netherlands). Morphometric parameters included bodylength (BL),

bodyweight (BW), eye diameters horizontal and vertical (EDh, EDv) and pectoral fin length (PFL). With these measurements we determined:

- Fulton's condition factor K using formula (2)
- The eye index according to Pankhurst (1982) EI using formula (3)
- The pectoral fin length index according to Durif et al. (2005) PFLI using formula (4)
- The silver index (SI) according to Durif et al. (2005) based on BL, BW, ED and PFL.

Withdrawal of blood (500µl) was performed in the dorsal aorta in the tail with heparin flushed (10.000 IU/ml) 1 ml syringes which were immediately placed on ice. Hematocrit (Hct) values were measured in 9 µl whole blood samples in triplo using a micro-centrifuge (Bayer, F.R.G.). Haemoglobin (Hb) content in 10 µl was determined in duplo by a spectrophotometer (LS50B, Perkin Elmer) measuring the absorbance at a fixed λ of 550 nm using the MPR 3 kit (1 ml, Roche Diagnostics GmbH). The MCHC (Mean Cellular Haemoglobin Content) was calculated dividing Hb by Hct.

**Table 1** Used formulas.

1. $\dot{M}O_2 = 127* \Delta[O_2]/\Delta t$ ($mgO_2/kg/h$), where: $\Delta[O_2]/\Delta t$ is the decrease of the oxygen content per hour
2. $K= 100* BW/BL^3$
3. $EI= 100* ((EDh+EDv)/4)^2 \pi /10*BL)$
4. $PFI= 100* PFL/BL$

*Experimental protocols*

Eels were introduced into the swim tunnels at least two days before the experiment started. Trials were performed at either fresh water (FW) or artificial salt water (SW). Salinity and water temperature were measured just before every trial. Salinity values of SW during experiments were 32±1 ppt. Water temperature values during experiments were 18±1ºC. Oxygen electrodes were callibrated with natriumsulfite (0%) and air (100%).

The swim fitness protocol consisted of 7 daily experimental trials: 2 speed tests and 5 endurance tests. On day 1, eels were subjected to a first speed test. Eels started to swim at a *U* of 0.5 m/s for 2 h. During these 2 h, we measured the decreasing oxygen content in the tunnel over the first 1.5 h, thereafter the tunnel was rinsed for 0.5 h. After these 2 h at 0.5 m/s, *U* was raised with 0.1 m/s to 0.6 m/s for 2 h, again measuring oxygen consumption over the first 1.5 h. Subsequently, this was repeated with steps of 0.1 m/s for a *U* up to 1.0 m/s. From day 2 to 6 eels were subjected to endurance tests. During endurance tests, the oxygen content in the tunnels was measured continuously. Rinsing occurred automatically so eels were swimming between 75 up to 85% air saturation. On day 2, eels swam at 0.5 m/s for 12 h. On day 3, eels swam 1 h at 0.5 m/s followed by 11 h at 0.6 m/s. On day 4, eels were subjected to1 h steps of 0.1 m/s up to swimming for 10 h at 0.7 m/s. Subsequenlty on day 5, 1 h steps of 0.1 m/s up to swimming for 9 h at 0.8 m/s. Finally, on day 6, 1 h steps of 0.1 m/s up to swimming for 8 h at 0.9 m/s. On day 7, the protocol was finished with a second speed test with the purpose to quantify conditioning effects during the experimental period.

When fish fatigued during trials, the flow was lowered to 0.1 m/s. This speed was considered as resting since eels had the choice either to swim or to rest while mixing of the

water in the tunnel was sufficient. Oxygen consumption in a resting state was measured for a period of 3-4 h.

*Swim parameters*

To characterise swim capacity and efficiency we derived five parameters as illustrated in Figure 1:

1) Oxygen consumption in rest ($\dot{M}O_{2\ rest}$) in mg $O_2$ per hour and per kg eel,
2) The critical swim speed ($U_{crit}$) calculated according to Brett (1964),
3) Maximum aerobic oxygen consumption ($\dot{M}O_{2\ max}$) in mg $O_2$ per hour per kg eel,
4) The optimum swim speed ($U_{opt}$) or the speed at which eels swim most efficient by and the amount of work per distance reaches a minimum (Tucker, 1970),
5) The cost of transport (COT) at optimum swim speeds in mg $O_2$ per km per kg eel.

The optimum swim speed was determined by plotting a polynomial trendline through COT values vs. swim speeds per individual eel. The point on this trendline with the lowest COT was considered the optimum swim speed (Fig. 1) and was calculated by equaling the derative of the function of the polynomial trendline to zero. By filling in this value again in the function of the polynomial trendline, the corresponding COT could be obtained.

*Otolithometry*

On the day after the last speedtest, the eels were removed from the swim tunnels, anaesthetized and sacrificed by decapitation. Otoliths (sagitta) of the wild eels were removed and collected for age estimation. For farmed eels, this procedure was not necessary since the duration of their residence at the farm was known. The age estimation was carried out in the laboratory of Cemagref, Bordeaux, France by otolithometry according to the method described by Daverat (2005a). After their extraction, otoliths were cleaned of all organic matter in distilled water, dried with ethanol, and then stored in eppendorf tubes. The otoliths were later embedded in synthetic resin (Synolithe), then polished to the nucleus with a polishing wheel (Streuers Rotopol-35) using 2 different grits of sandpaper (1200 and 2400). Fine polishing was done by hand with $Al_2O_3$ (1µm grain) on a polishing cloth. Etching was done using 10% EDTA. A drop of this solution was applied on the mold for a duration of 15 minutes. The otoliths were then rinsed with distilled water and stored in dry conditions. Yearly increments were revealed by staining with a drop of 5% Toluidine blue on the otolith and letting it dry. Growth rings were then counted under a microscope. The age of each eel was determined by the number of increments starting from the nucleus which was considered as year 1 of the eel's life.

*Statistics*

Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov tests. With a univariate general linear model (GLM), analysis of covariance (ANCOVA) was performed on log transformed data in search for group effects in swim parameters with BL and BW as cofactors. In case of occurrence of significant group effects, ANOVA with post-hoc Bonferroni test was performed to specify the effects between particular groups. Pearson tests were performed for correlation analysis between BL, BW, EI and swim parameters. For comparison of general parameters between groups (table 2) and of swim parameters between speed and endurance trials, t-tests were used

either paired for values of the same eels or unpaired for values of different eels. All statistical analysis were performed in SPSS 10.0 for Windows.

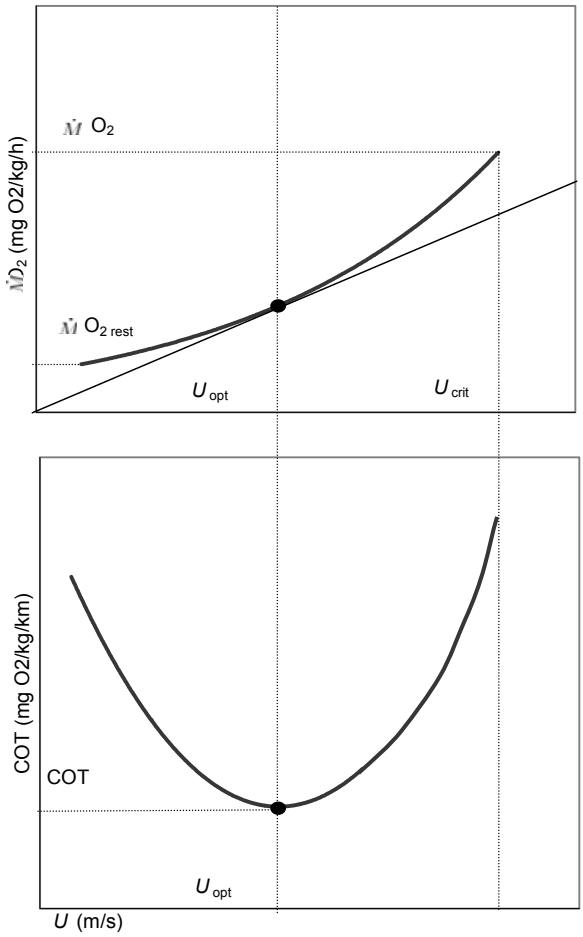


**Figure 1** Measured and theoretically derived swim parameters. On the y-axis is given the oxygen consumption $\dot{M}O_2$ and determined were the $\dot{M}O_2$ in rest and the maximum $\dot{M}O_2$. On the x-axis is given the swim speed and determined were the optimum swim speed $U_{opt}$ and the critical swim speed $U_{crit}$. At the $U_{opt}$, the cost of transport (COT) is lowest (*see text for detailed explanation*).

CHAPTER 2

RESULTS

*Experimental eels*

All farmed eels arrived as glass eels and spent 1.7 years at the farm (Table 2). They had eye indices ≥ 9.6 and were therefore all considered silver according to Pankhurst (1982). According to the silver index of Durif et al. (2005), only one was premigrant (stage 3) while the rest was migrant (stage 4-5). The wild eels from the salt water habitat Lake Grevelingen (n=19) were considerably older than the farmed eels with 11 ± 3 years old in a wide range between 7 and 20. They were also significantly larger ($P<0.01$) with 79 ± 5 cm, 949 ± 156 g ($P<0.05$) and had a significantly lower condition factor ($P<0.01$) of 0.19 ± 0.02 (Table 2). All eels were silver and pre-migratory or migratory (stage 3 or 4). Hct was significantly ($P<0.05$) lower than in farmed eels with 24.4 ± 7.3%. Hb was significantly ($P<0.01$) lower with 4.52 ± 1.62 mM and also the resulting MCHC was found significantly ($P<0.01$) lower with of 0.19 ± 0.03 mM, possibly by cell swelling. The wild eels from the fresh water habitat River Loire (n=20) were even older ($P<0.01$) with 16 ± 4 years old in a wide range between 10 and 28. All Loire eels were silver but not all were migratory since two silver eels were still pre-migrants at SI stage 3 (Durif et al., 2005). The pectoral fins of Loire eels were significantly ($P<0.01$) larger than the farmed eels. Hct was significantly ($P<0.01$) higher than in farmed eels with 39.6 ± 5.7%. However, Hb was lower with 5.52 ± 0.70 mM. The MCHC was found significantly ($P<0.01$) lower with of 0.14 ± 0.01 mM.

In general, the wild migratory silver eels were much older than the farmed silver eels. They were also 8 to 11 cm longer and were about 150 g heavier. Their condition factor was however lower. The wild migratory eels had significantly longer pectoral fins ($P<0.01$). Overall, the MCHC was much lower ($P<0.001$) in the wild migratory eels.

*Swimming of experimental eels and measuring their oxygen consumption*

All experimental eels were females so no sex differences could be observed. The eels appeared to be in good health. No eels died during experimental periods except for some accidents. Incidentally, a few eels did not swim at the beginning of an experiment, but those were easily stimulated by shining a flashlight on their eyes or by knocking on the swimtunnel. Also incidentally, some eels were "brushing the wall" (Brett, 1964), swimming close to the wall of the swim tunnel in search for a lower flow in the first 2 cm from the swim tunnel wall (Van den Thillart et al., 2004), however this only occurred with some smaller eels (<60 cm) when swimming at high speeds (0.9-1.0 m/s). Data of those eels were rejected for analysis. Eels mainly swam a few cm below the center in the front part of the tunnel near the grid. We did not observe any irregularities in swimming of eels at any size (up to 1 m).

*Speed and endurance of farmed eels (SW)*

The $O_2$ content in the swim-tunnels showed a gradual decrease during swimming of experimental eels. Figure 2 shows an example during swimming of a single experimental farmed eel during a speed test. With every increment, the slope becomes steeper indicating increase of $\dot{M}O_2$. The gradual decrease shows the ability to stabilise during 2 h swimming for each *U*. Endurance test results showed that eels were able to stabilise their $\dot{M}O_2$ rates during 12 h swim periods. Figure 3 shows a typical example of an endurance test for a

**Table 2** Means, standard deviations (SD) and their range of parameters measured on experimental eels of all groups before the start of swim trials and their age either known or estimated by otolithometry.

| | | Farm, small (SW) | Farm, large (SW) | Farm, large (FW) | Lake Grevelingen (SW) | Loire (FW) |
|---|---|---|---|---|---|---|
| age | mean | 1.7 | 1.7 | 1.7 | 11 | 16 |
| | SD | | | | 3 | 4 |
| | range | | | | 7-20 | 10-28 |
| BL | mean | 64 | 71 | 71 | 79 | 82 |
| | SD | 3 | 4 | 4 | 5 | 6 |
| | range | 60-70 | 64-80 | 63-75 | 69-88 | 70-96 |
| BW | mean | 536 | 799 | 824 | 949 | 1018 |
| | SD | 81 | 129 | 135 | 156 | 253 |
| | range | 409-648 | 659-1191 | 560-1062 | 635-1238 | 668-1473 |
| K | mean | 0.20 | 0.23 | 0.23 | 0.19 | 0.18 |
| | SD | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| | range | 0.17-0.23 | 0.20-0.29 | 0.19-0.28 | 0.16-0.24 | 0.15-0.20 |
| EI | mean | | | 11.0 | 11.7* | 11.2 |
| | SD | | | 1.0 | 1.9 | 1.3 |
| | range | | | 9.6-13.3 | 8.2-14.7 | 8.7-13.5 |
| PFI | mean | | | 3.88 | 4.52 | 4.87 |
| | SD | | | 0.46 | 0.36 | 0.33 |
| | range | | | 3.27-4.89 | 4.08-5.04 | 4.15-5.25 |
| SI | mean | | | 4.0 | 3.9* | 4.3 |
| | SD | | | 0.4 | 0.32 | 0.7 |
| | range | | | 3-5 | 3-4 | 3-5 |
| Hct | mean | 29.1 | 31.2 | 29.8 | 24.4 | 39.6 |
| | SD | 6.6 | 3.7 | 6.5 | 7.3 | 5.7 |
| | range | 17.5-45.2 | 23.3-40.3 | 9.5-38.6 | 9.5-36.5 | 27.5-46.6 |
| Hb | mean | | | 7.49 | 4.52 | 5.52 |
| | SD | | | 1.24 | 1.62 | 0.70 |
| | range | | | 4.97-9.54 | 2.03-7.76 | 4.05-6.44 |
| MCHC | mean | | | 0.26 | 0.19 | 0.14 |
| | SD | | | 0.07 | 0.03 | 0.01 |
| | range | | | 0.20-0.52 | 0.15-0.23 | 0.12-0.17 |

* data taken from similar sized eels (n=13) from the same batch for comparison

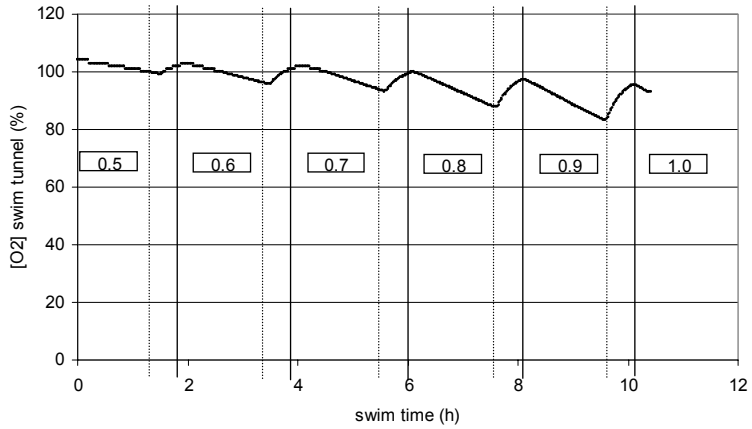


**Figure 2** The $[O_2]$ in a swim tunnel as percentage of air saturation expressed against the swim time of an experimental farmed eel swimming (SW) in the tunnel during a speed test. During each *U* (solid lines), valves were closed for 1.5 h and opened for 0.5 h (dashed lines) to rinse the system. The downward slopes of $[O_2]$ at a specific *U* indicates $\dot{M}O_2$. Slopes were gradual without irregularities indicating steady consumption rates. Slopes became steeper during increasing *U*s showing increased $\dot{M}O_2$. This particular eel fatigued after swimming 17 minutes at 1.0 m/s.

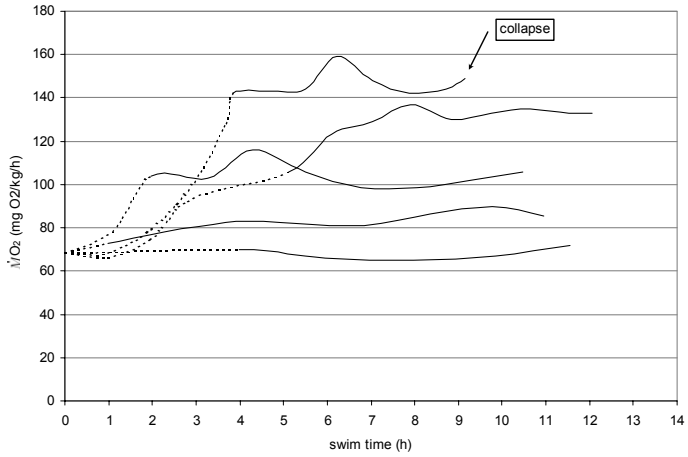


**Figure 3** Typical experiment of an endurance test ($\dot{M}O_2$ profiles vs. swim time). A female silver eel (856 g, 73 cm) was exposed to step wise increasing water flow, the first day to 0.5 m/s and the fifth day to 0.9 m/s. The start speed was 0.5 m/s, the increments 0.1 m/s per hour. Each run lasted 12 hours, overnight the eels were rested. The starting point on the x-axis is the group average. Dashed lines indicate incremental *U* steps. $\dot{M}O_2$ rates were higher at every speed. Once reached the targeted *U*, $\dot{M}O_2$ rates were stable within a range of 20 mg $O_2$/kg/h.

farmed silver eel. $\dot{M}O_2$ rates per speed stayed well within a range of 20 mg $O_2$/kg/h. $\dot{M}O_2$ rates of the two grouped of farmed eels swimming in SW were pooled and expressed vs. *U* (Table 3). Paired observations are given of the same experimental eels for the two speed tests and the five endurance tests. Results of the speed tests indicated a conditioning effect, as during the second speed test eels fatigued later. Results for speed tests and endurance tests were similar. Only at a *U* of 0.8 m/s we found a significant difference in $\dot{M}O_2$ of the first speed test and the endurance tests. This difference was not significant between the second speed test and the endurance tests showing that this was due to conditioning during the experiment. In Table 3 we also expressed COT vs. *U*. COTs were rather constant between 45 and 53 mg $O_2$/kg/km at *U*s of 0.5 up to 0.9 m/s. Only 10 out of the total 42 farmed eels were able to swim at 1.0 m/s for longer periods during the speed tests. COTs at these speeds were higher at 58 and 62 mg $O_2$/kg/km. Since results for speed and endurance tests were similar for these eels, we subjected other groups only to a single speed test.

**Table 3** Pooled mean $\dot{M}O_2$ and COT data of farmed eels (SW) during the various *U*s of speed test 1, 2 and the endurance tests. Comparison shows that $\dot{M}O_2$ data of the first speed test and endurance tests are similar. Only at 0.8 m/s, values were different probably due to conditioning. COTs were found between 45 and 53 mg $O_2$/kg/km and constant for speeds between 0.5 and 0.9 m/s. The asterix marks a significant (P<0.05) difference between the first speed test and the endurance test.

| | | | swim speed (m/s) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **0.5** | **0.6** | **0.7** | **0.8** | **0.9** | **1.0** |
| $\dot{M}O_2$ | **speed test 1** | **mean** | 89 | 101 | 125 | 140 | 147 | 161 |
| | | **SD** | 15 | 14 | 14 | 16 | 25 | 17 |
| | | **n** | 35 | 36 | 30 | 17 | 8 | 3 |
| | **speed test 2** | **mean** | 87 | 100 | 126 | 150 | 167 | 173 |
| | | **SD** | 15 | 17 | 17 | 21 | 21 | 17 |
| | | **n** | 40 | 41 | 41 | 39 | 31 | 7 |
| | **endurance tests** | **mean** | 85 | 104 | 129 | 153* | 161 | |
| | | **SD** | 12 | 12 | 14 | 19 | 23 | |
| | | **n** | 39 | 42 | 39 | 32 | 11 | |
| **COT** | **speed test 1** | **mean** | 49 | 47 | 50 | 49 | 45 | 58 |
| | | **SD** | 8 | 7 | 6 | 6 | 8 | 6 |
| | | **n** | 35 | 36 | 30 | 17 | 8 | 3 |
| | **speed test 2** | **mean** | 49 | 46 | 50 | 52 | 52 | 62 |
| | | **SD** | 8 | 8 | 7 | 7 | 6 | 6 |
| | | **n** | 40 | 41 | 41 | 39 | 31 | 7 |
| | **endurance tests** | **mean** | 47 | 48 | 50 | 53 | 50 | |
| | | **SD** | 6 | 6 | 10 | 7 | 7 | |
| | | **n** | 39 | 42 | 40 | 32 | 11 | |

*Swim characteristics of farmed eels (SW)*

$\dot{M}O_{2\ rest}$ of the smaller farmed eels was on average 38 ± 5 mg $O_2$/kg/h with a range between 27 to 48 (Table 4). The mean $U_{crit}$ during the first speedtest was 1.24 ± 0.15 BL/s or 0.80 ± 0.08 m/s (Table 4). The mean $U_{crit}$ during the second speedtest was 1.31 ± 0.13 BL/s or 0.84 ± 0.06 m/s. This difference was significant (P<0.05) and expressed a mean training effect of 5.6%. For endurance trials a $U_{crit}$ value was found of 1.25 ± 0.12 BL/s. $\dot{M}O_{2\ max}$ values were often reached at speeds below $U_{crit}$. Swimming near or at $U_{crit}$ involved apparantly a large anaerobic component creating an oxygen debt. $\dot{M}O_{2\ max}$ was on average 136 ± 23 mg $O_2$/kg/h for the first speed test, 165 ± 32 mg $O_2$/kg/h for the second speed test

**Table 4** Means, standard deviations (SD) and their range of swim parameters measured on experimental eels of all groups a to e. For both groups of farmed eels swimming in SW, two speed tests and an endurance test were performed, for other groups just one speed test. The given COT is the lowest at the optimum swim speed. Bold are statistic differences ($P<0.05$; Table 5) between groups as indicated.

| | | a) Farm, small, SW (20) | | | b) Farm, large, SW (22) | | | c) Farm, large, FW (20) | d) Lake Grevelingen SW (19) | e) Loire FW (20) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | speed 1 | speed 2 | endurance | speed 1 | speed 2 | endurance | speed 1 | speed 1 | speed 1 |
| $U_{crit}$ | mean | **1.24 (ae)** | 1.31 | 1.25 | 1.05 | 1.11 | 1.13 | **1.15 (ce)** | 0.91 | **0.81 (ace)** |
| (BL/s) | SD | 0.15 | 0.13 | 0.12 | 0.12 | 0.13 | 0.12 | 0.20 | 0.20 | 0.24 |
| | range | 1.02-1.54 | 1.01-1.50 | 1.04-1.48 | 0.83-1.32 | 0.82-1.32 | 0.89-1.34 | 0.69-1.43 | 0.48-1.21 | 0.48-1.29 |
| $U_{crit}$ | mean | 0.80 | 0.84 | 0.80 | 0.74 | 0.78 | 0.79 | 0.81 | 0.71 | 0.66 |
| (m/s) | SD | 0.08 | 0.06 | 0.06 | 0.08 | 0.08 | 0.07 | 0.13 | 0.14 | 0.18 |
| | range | 0.71-0.93 | 0.71-0.92 | 0.73-0.90 | 0.62-0.91 | 0.61-0.93 | 0.66-0.90 | 0.51-0.94 | 0.42-0.96 | 0.40-0.91 |
| $U_{opt}$ | mean | 1.02 | 0.98 | 0.92 | 0.96 | 0.85 | 0.82 | 0.94 | 0.77 | 0.74 |
| (BL/s) | SD | 0.16 | 0.13 | 0.09 | 0.23 | 0.07 | 0.06 | 0.11 | 0.15 | 0.09 |
| | range | 0.74-1.41 | 0.82-1.37 | 0.79-1.07 | 0.74-1.69 | 0.74-1.00 | 0.70-0.95 | 0.70-1.15 | 0.56-1.02 | 0.58-0.87 |
| $U_{opt}$ | mean | 0.65 | 0.63 | 0.59 | 0.68 | 0.60 | 0.58 | 0.66 | 0.62 | 0.61 |
| (m/s) | SD | 0.10 | 0.06 | 0.04 | 0.18 | 0.05 | 0.03 | 0.06 | 0.12 | 0.05 |
| | range | 0.50-0.89 | 0.54-0.82 | 0.53-0.66 | 0.52-1.22 | 0.52-0.72 | 0.52-0.65 | 0.49-0.74 | 0.45-0.82 | 0.51-0.69 |
| $\dot{M}O_{2\ rest}$ | mean | 38 | | | **35 (be)** | | | 38 | **35 (de)** | **43 (bde)** |
| (mg/kg/h) | SD | 5 | | | 4 | | | 5 | 8 | 13 |
| | range | 27-48 | | | 28-41 | | | 28-49 | 26-50 | 31-77 |
| $\dot{M}O_{2\ max}$ | mean | 136 | 165 | 158 | 134 | 159 | 148 | 130 | 152 | 138 |
| (mg/kg/h) | SD | 23 | 32 | 26 | 16 | 16 | 18 | 26 | 39 | 38 |
| | range | 105-175 | 93-218 | 115-212 | 106-161 | 122-189 | 105-178 | 93-191 | 80-224 | 91-230 |
| COT | mean | 43 | 45 | 49 | **46 (bc)** | 45 | 45 | **37 (bcd)** | **50 (cd)** | 44 |
| (mg/kg/km) | SD | 5 | 6 | 6 | 8 | 5 | 4 | 5 | 8 | 11 |
| | range | 32-52 | 30-56 | 35-62 | 23-57 | 37-56 | 37-54 | 23-43 | 36-65 | 31-69 |

and 158 ± 26 mg $O_2$/kg/h for endurance tests (Table 4). Maxima were found up to even 218 mg $O_2$/kg/h. The mean $U_{opt}$ during the first speedtest was 1.02 ± 0.16 BL/s or 0.65 ± 0.10 m/s (Table 4). The mean $U_{opt}$ during the second speedtest was similar with 0.98 ± 0.13 BL/s or 0.63 ± 0.06 m/s. The mean $U_{opt}$ for endurance trials was found slightly lower with 0.92 ± 0.09 BL/s or 0.59 ± 0.04 m/s, significant only vs. the second speedtest ($P<0.05$). COT values at $U_{opt}$ were similar for both speed tests with 43 ± 5 and 45 ± 6 mg $O_2$/kg/km. Higher values ($P<0.01$) were found at the endurance trials with 49 ± 6 mg $O_2$/kg/km.

$\dot{M}O_{2\ rest}$ of the larger farmed eels was on average 35 ± 4 mg $O_2$/kg/h with a range between 28 to 41 (Table 4). The mean $U_{crit}$ during the first speedtest was 1.05 ± 0.12 BL/s or 0.74 ± 0.08 m/s (Table 4). The mean $U_{crit}$ during the second speedtest was 1.11 ± 0.13 BL/s or 0.78 ± 0.08 m/s. This difference was significant ($P<0.05$) and expressed a training effect of 5.7%, similar to the training effect found for smaller eels. For endurance trials a $U_{crit}$ value was found of 1.13 ± 0.12 BL/s. $\dot{M}O_{2\ max}$ was on average 134 ± 16 mg $O_2$/kg/h for the first speed test, 159 ± 16 mg $O_2$/kg/h for the second speed test and 148 ± 18 mg $O_2$/kg/h for endurance tests (Table 4). Maxima were found up to 189 mg $O_2$/kg/h. The mean $U_{opt}$ during the first speedtest was 0.96 ± 0.23 BL/s or 0.68 ± 0.18 m/s (Table 4). The mean $U_{opt}$ during the second speedtest was lower ($P<0.05$) with 0.85 ± 0.07 BL/s or 0.60 ± 0.05 m/s. The mean $U_{opt}$ for endurance trials was also lower ($p<0.05$) with 0.82 ± 0.06 BL/s or 0.58 ± 0.03 m/s. COT values at $U_{opt}$ were similar for speedtests with 46 ± 8 and 45 ± 5 mg $O_2$/kg/km and for endurance trials with 45 ± 4 mg $O_2$/kg/km.

The group of large farmed eels was significantly larger ($P<0.0001$), heavier ($P<0.0001$) and had a higher condition factor ($P<0.0001$) than the group of smaller farmed eels (Table 2). Therefore the two groups could be used to study the size effects.
Group-wise comparison by ANCOVA showed that the $\dot{M}O_{2\ rest}$ and $U_{crit}$ of the larger eels was lower but not significantly different (Table 5). On average the performance of larger eels was 9.6% lower for endurance tests and 15.3% for speed tests (resp. 1.4 and 2.2% per cm BL). Mean $U_{opt}$ values were slightly lower for larger eels but also not significantly different. Absolute values were similar. COT values were slightly higher for larger eels but not significantly.

Individual comparison by correlation analysis (Table 6) showed that BL and BW significantly correlated with all absolute values for swim parameters. Positive correlations existed between size and $\dot{M}O_{2\ rest}$ (mg $O_2$/h; $P<0.0001$), $\dot{M}O_{2\ max}$ (mg $O_2$/h; $P<0.0001$), $U_{opt}$ (m/s; $P<0.05$) and COT (mg $O_2$/km; $P<0.0001$). A negative correlation existed between size and $U_{crit}$ (m/s; $P\leq0.05$). Relatively, BL and BW significantly correlated negatively with $\dot{M}O_{2\ rest}$ (mg $O_2$/kg/h; $P<0.01$) and $U_{crit}$ (BL/s; $P<0.0001$; Table 6). The negative correlation with the relative $\dot{M}O_{2\ rest}$ expresses the underestimation of values for smaller eels when extrapolating to 1 kg or, in other words, the metabolic weight of a smaller eel is larger than a larger eel. This relation was found in all groups (Fig. 3). The negative correlations between size and $U_{crit}$ shows that larger eels not only reach relatively lower $U_{crit}$ but also absolutely lower $U_{crit}$. Thus, small eels were able to swim significantly faster.

**Table 5** Significant differences of swim parameters between groups by a) ANCOVA showing overall group effects and effects of BL and BW, followed by b) ANOVA showing the significant effects between particular groups. Values are P values and ns= not significant.

| ANCOVA | group | BL | BW |
|---|---|---|---|
| $\dot{M}O_{2\ rest}$** | 0.01 | ns | <0.001 |
| $U_{crit}$* | 0.001 | ns | ns |
| $\dot{M}O_{2\ max}$** | 0.034 | ns | <0.001 |
| $U_{opt}$* | ns | ns | ns |
| COT** | <0.001 | ns | <0.001 |

| | ANOVA | Farm, large (SW) | Farm, large (FW) | Lake Grev. (SW) | Loire (FW) |
|---|---|---|---|---|---|
| Farm, small (SW) | $\dot{M}O_{2\ rest}$** | ns | ns | ns | ns |
| | $U_{crit}$* | ns | ns | ns | **0.005** |
| | $\dot{M}O_{2\ max}$** | ns | ns | ns | ns |
| | $U_{opt}$* | ns | ns | ns | ns |
| | COT** | ns | ns | ns | ns |
| Farm, large (SW) | $\dot{M}O_{2\ rest}$** | | ns | ns | **0.02** |
| | $U_{crit}$* | | ns | ns | ns |
| | $\dot{M}O_{2\ max}$** | | ns | ns | ns |
| | $U_{opt}$* | | ns | ns | ns |
| | COT** | | **0.008** | ns | ns |
| Farm, large (FW) | $\dot{M}O_{2\ rest}$** | | | ns | ns |
| | $U_{crit}$* | | | ns | **0.004** |
| | $\dot{M}O_{2\ max}$** | | | ns | ns |
| | $U_{opt}$* | | | ns | ns |
| | COT** | | | **<0.001** | ns |
| Lake Grev. (SW) | $\dot{M}O_{2\ rest}$** | | | | **0.01** |
| | $U_{crit}$* | | | | ns |
| | $\dot{M}O_{2\ max}$** | | | | ns |
| | $U_{opt}$* | | | | ns |
| | COT** | | | | ns |

* absolute values compared because no effects length and weight ($U_{crit}$: m/s, $U_{opt}$: m/s)

** relative value compared because effect weight (COT: mg/kg/km)

*Swimming in SW vs FW*

$\dot{M}O_{2\ rest}$ of farmed eels in FW was on average 38 ± 5 mg $O_2$/kg/h with a range between 28 to 49 (Table 4). A mean $U_{crit}$ was found of 1.15 ± 0.20 BL/s or 0.81 ± 0.13 m/s (Table 4). $\dot{M}O_{2\ max}$ was on average 130 ± 26 mg $O_2$/kg/h (Table 4). A mean $U_{opt}$ was found of 0.94 ±

Results were compared with results of the larger farmed eels swimming in SW. Both groups were of the same origin and very similar in age, morphological parameters and Hct and therefore considered as completely comparable (Table 2). The $\dot{M}O_{2\ rest}$ was higher but not significantly different for eels in fresh water (Table 4). The $U_{crit}$ was found higher with 0.10 BL/s or 7 cm/s (9.5%) in fresh water but not significantly different. $\dot{M}O_{2\ max}$ was found similar. The $U_{opt}$ was also found similar but the COT at these speeds was found significantly lower for swimming in FW (P<0.01; ANCOVA Table 5).

**Table 6** Results of the swim fitness tests of Lake Grevelingen and River Loire groups were compared. $\dot{M}O_{2\ rest}$ was significantly higher but not for the Loire eels in Correlations between BL, BW and swim parameters, either as absolute (A) or relative values (R).

| | | $\dot{M}O_{2\ max}$ | $\dot{M}O_{2\ rest}$ | COT | $U_{crit}$ | $U_{opt}$ |
|---|---|---|---|---|---|---|
| ABSOLUTE | | | | | | |
| BL | corr. | **0.781** | **0.761** | **0.743** | -0.257 | **0.293** |
| | P | **0.000** | **0.000** | **0.000** | 0.052 | **0.041** |
| | n | **36** | **40** | **36** | 41 | **36** |
| BW | corr. | **0.859** | **0.863** | **0.794** | **-0.294** | **0.359** |
| | P | **0.000** | **0.000** | **0.000** | **0.031** | **0.016** |
| | n | **36** | **40** | **36** | **41** | **36** |
| RELATIVE | | | | | | |
| BL | corr. | -0.197 | **-0.440** | **-0.064** | **-0.673** | -0.051 |
| | P | 0.124 | **0.02** | **0.356** | **0.000** | 0.383 |
| | n | 36 | **40** | **36** | **41** | 36 |
| BW | corr. | -0.182 | **-0.450** | **-0.083** | **-0.643** | 0.042 |
| | P | 0.144 | **0.002** | **0.315** | **0.000** | 0.404 |
| | n | 36 | **40** | **36** | **41** | 36 |

*Swimming of Lake Grevelingen (SW) and River Loire (FW) migratory eels*

$\dot{M}O_{2\ rest}$ of Lake Grevelingen eels in SW was on average 35 ± 8 mg $O_2$/kg/h with a range between 26 to 50 (Table 4). A mean $U_{crit}$ was found of 0.91 ± 0.20 BL/s or 0.71 ± 0.14 m/s (Table 4). The $\dot{M}O_{2\ max}$ was on average 152 ± 39 mg $O_2$/kg/h (Table 4). A mean $U_{opt}$ was found of 0.77 ± 0.15 BL/s or 0.62 ± 0.12 m/s (Table 4). COT values were 50 ± 8 mg $O_2$/kg/km.

$\dot{M}O_{2\ rest}$ of River Loire eels in FW was on average 43 ± 13 mg $O_2$/kg/h with a wide range between 31 to 77 (Table 4). A mean $U_{crit}$ was found of 0.81 ± 0.24 BL/s or 0.66 ± 0.18 m/s (Table 4). $\dot{M}O_{2\ max}$ was on average 138 ± 38 mg $O_2$/kg/h (Table 4). A mean $U_{opt}$ was found of 0.74 ± 0.09 BL/s or 0.61 ± 0.05 m/s (Table 4). COT values were 44 ± 11 mg $O_2$/kg/km.

Results of the swim fitness tests of Lake Grevelingen and River Loire groups were compared. $\dot{M}O_{2\ rest}$ was significantly higher but not for the Loire eels in fresh water (P=0.01; Table 4; Table 5). The $U_{crit}$ was found lower in Loire eels in fresh water but not significantly with 0.10 BL/s or 5 cm/s (11%), comparable with the 9.5% found in farmed eels in FW. $\dot{M}O_{2\ max}$ was found lower in Loire eels but not significantly. The $U_{opt}$ was found similar and the COT was found lower in Loire eels but not significantly (Table 5).

*Swimming of farmed eels vs wild migratory eels*

Results of the swim fitness tests between the large farmed eels and the wild migratory eels were compared. For SW and FW identical differences were observed. The wild migratory eels had lower $U_{crit}$,, lower $U_{opt}$ and higher COT. ANCOVA showed however effects of bodyweight on $\dot{M}O_{2\ rest}$,, $\dot{M}O_{2\ max}$ and COT. By eliminating effects of BW and effects of swimming in SW or FW, only $U_{crit}$ was found significantly different between farmed and wild eels. Differences between farmed and wild eels were more pronounced in fresh water.

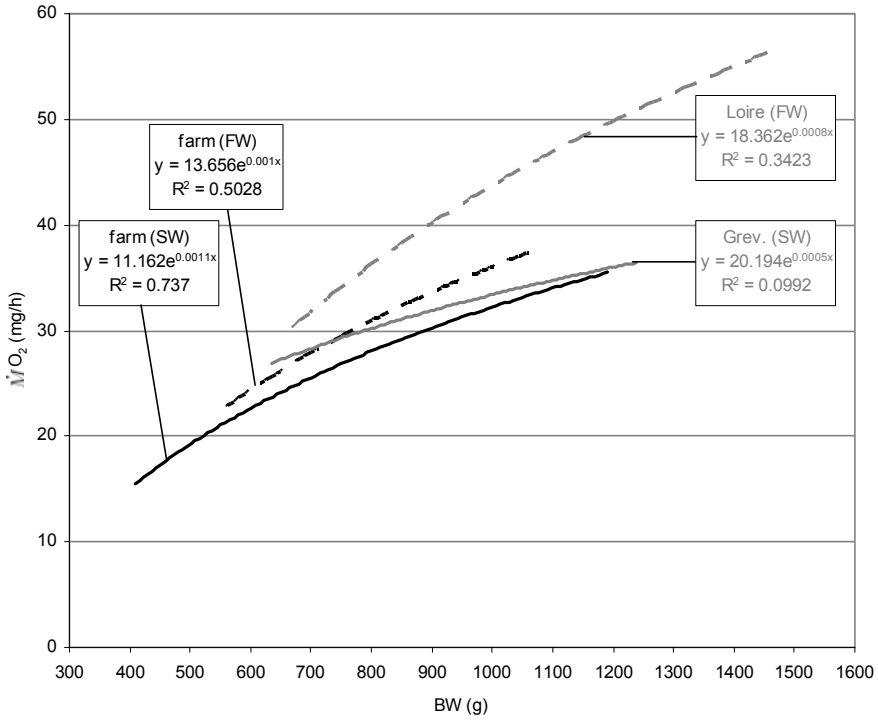


**Figure 4** Trendlines of $\dot{M}O_{2\ rest}$ vs. BW for experimental eels from the farm in SW or FW and wild migratory eels in SW or FW.

## DISCUSSION

*Swim fitness of silver eels*

This study has been the first study testing the swim capacity and efficiency of large female silver eels on a large scale. The enormous amount of 500,000 $\dot{M}O_2$-datapoints was collected for analysis. Earlier swim experiments were performed only on small eels (< 60 cm; Davidson, 1949; Schmidt-Nielsen, 1972; Webb, 1975; Tsukamoto et al., 1975; McCleave, 1980; Mitchell, 1989; Barbin & Krueger, 1994; Gilis, 1998; Langdon & Collins, 2000; Van Ginneken et al., 2002) and often in low numbers. At Leiden University we have a set-up of 22 Blazka swim tunnels. The swim performance of carp *Cyprinus carpio* and rainbow trout *Oncorhynchus mykiss* has been investigated in our tunnels (Van Dijk et al., 1993; Van Ginneken et al., 2005b) and provided similar results as reported in literature. Recent swim experiments with large eels mainly concerned long term simulated migration trials (Van Ginneken & Van den Thillart, 2000; Van den Thillart et al., 2004; Van Ginneken et al., 2005b).

In this study we found that silver eels showed similar swim performance during speed tests and endurance tests. We did not find a difference between swimming for 2 h and swimming for 12 h, except for minor conditioning effects. Farmed eels that were exercised at the second speed test fatigued later and showed about 5% higher $U_{crit}$. Also exercised rainbow trout *Oncorhynchus mykiss*, sockeye salmon *Oncorhynchus nerka* and coho salmon *Oncorhynchus kisutch* tend to fatigue later than unexercised individuals (reviewed by Beamish, 1978).Eels kept $\dot{M}O_2$ rates rather constant during swimming at all speeds, for 2 h as well as for 12 h periods. During 12 h swimming the rates stayed within a range of 20 mg $O_2$/kg/h (Fig. 3). The COTs at these *U*s between 0.5 and 0.9 m/s were also rather constant staying between 45 and 53 mg $O_2$/kg/km. This shows the ability to stabilise and maintain metabolic balance. Results of the speed test have therefore highly predictive value. This makes it possible to determine swim performance in a single speed test, thus according to a single day protocol. In this study, we have applied the speed test to measure the swim fitness of various groups of silver eels.

*High swim efficiency of silver eels*

We found that silver eels swam highly efficient. At optimum swim speeds of 0.58-0.68 m/s or 0.74-1.02 BL/s, COT values were found of 37-50 mg $O_2$/kg/km which are very low. In comparison, Videler (1993) reviewed results for 12 undulatory swimming fish species and reported optimum swim speeds of 0.18-0.51 m/s or 0.8-2.8 BL/s with corresponding COT values of 113-475 mg $O_2$/kg/km. From biomechanics it is known that for physical reasons locomotory specialisations are mutually exclusive, e.g. cruisers like silver eels necessarily have poor accelerating capabilities and vice versa (Gemballa, 2005). Indeed, we found that silver eels did not swim fast relatively to other species. Critical swim speeds were found between 0.81 up to 1.24 BL/s or 0.66 up to 0.81 m/s for the various groups of eels. Burst speeds are supposed not to be much higher in comparison with the 1.9 BL/s (1.14 m/s) found for a 60 cm eel swimming for 2-5 s in a swim tunnel (Blaxter & Dickson, 1959). In comparison with eel elvers, McCleave (1980) found burst speeds of 7.5 BL/s or 0.53 m/s. These values are comparable to another migrating species with an elongated body, the sea lamprey *Petromyzon marinus*. This species has critical swim speeds of 1.01 up to 1.34 BL/s or 0.82 up to 1.19 m/s (Almeida et al., 2005). Values of eels are low in comparison to cyprinid and salmonid species having prolonged swim speeds above 3 BL/s and mean burst speeds of 10 BL/s (reviewed by Videler, 1993). We can therefore conclude that silver eels are highly efficient cruisers with poor performance at high swim speeds. This indicates a high degree of locomotory specialisation in favour of cruising as a probable result of 60 million years of selection pressure on Darwinian fitness.

*Constraints of salinity and habitat on swim fitness*

Energy expenditure during exercise was higher in SW than in FW. The COT of farmed eels was found 20% higher when swimming in SW. Also wild eels showed a higher COT in SW although this effect could be due to the difference in origin. These results agree with the hypothesis that osmoregulation would require higher energy expenditure. Measurements in literature are scarce and do not provide a clear picture. Energy expended for swimming by rainbow trout *Oncorhynchus mykiss* and tilapia *Tilapia nilotica* was found to be independent of salinity. However, changes in metabolic rate did occur, suggesting that

overall performance would still be reduced (reviewed in Beamish, 1978). Morgan & Iwama (1998) did not find significantly different oxygen consumption rates in juvenile coho salmon *Oncorhynchus kisutch* after increase of salinity. Possibly the osmotic difference together with the permeability of the gills determines how much extra energy must be spend by the animal which may be species specific. Increase in oxygen consumption may also be explained by the environment that is natural for the specific life-history stage like suggested for salmonids by Morgan & Iwama (1991). Recently, Rankin et al. (2006) found that marine osmoregulation in eels indeed improves during silvering, as shown by a positive correlation between eye index and plasma osmolality at 36h after transfer to seawater, and is maintained until ovulation. However, experimental eels that were used for comparison between swimming in fresh and salt water in our study were all silver so this would not account (partly) for a rise of oxygen consumption.

The wild eels used in this study were much older than the farmed eels. The silver eels from Lake Grevelingen were on average 11 ± 3 years when migrating and younger and less silver than the eels from River Loire of 16 ± 4 years. Surprisingly, these wild eels were not in more advanced silver stages in comparison with the farmed eels both according to the indices of Pankhurst (1982) and Durif (2005). They had however much longer pectoral fins than the farmed eels. Although we expected that the wild eels would be better cruisers than the farmed eels, we found trends for even lower $U_{opt}$ (8-9% for m/s) and higher COT (9-20%). The $U_{crit}$ was found significantly lower (4-19% for m/s). These findings accounted for silver eels of both locations. Evidently, the wild eels had a lower condition that might reflect a difference in trophic quality. Firstly, fat percentages in wild eels were lower as indicated by K and was observed during sampling. A higher fat percentage results in an easier maintance of neutral buoyancy (Seibel, 2005). Secondly, silver eels from the Loire River, which scored the lowest $U_{crit}$ and $U_{opt}$ values, suffered from severe infection with the swim-bladder parasite *Anguillicola crassus*. This suggests that *A. crassus* impairs of swim capacity and efficiency of silver eels.

*Implications for reproductive migration to the Sargasso*

Optimum swim speeds for wild silver eels in SW were 0.77 BL/s or 0.62 m/s. When we assume that silver eels cruise at optimum swim speeds, these speeds are much higher than the generally assumed cruise speed of 0.5 BL/s. These optimum swim speeds agree with sustained cruising speeds between 0.6 – 0.9 BL/s found for 11 silver eels (69 to 96 cm) tracked in the North Sea by Tesch (1974; reviewed by Beamish, 1978). As for other tracking studies on migrating silver eels, optimum swim speeds only correspond to the fastest migration speeds found in the wild (Tesch, 1978, 1989, 2003; Tesch et al., 1991; McCleave & Arnold, 1999; Jellyman & Tsukamoto, 2002). If female silver eels would cruise at optimum swim speeds, they would travel 3.5 months to the Sargasso instead of the generally assumed 6 months (Tesch, 1977). Consequently, female migration may start later and/or spawning may start earlier (reviewed by McCleave, 2003).

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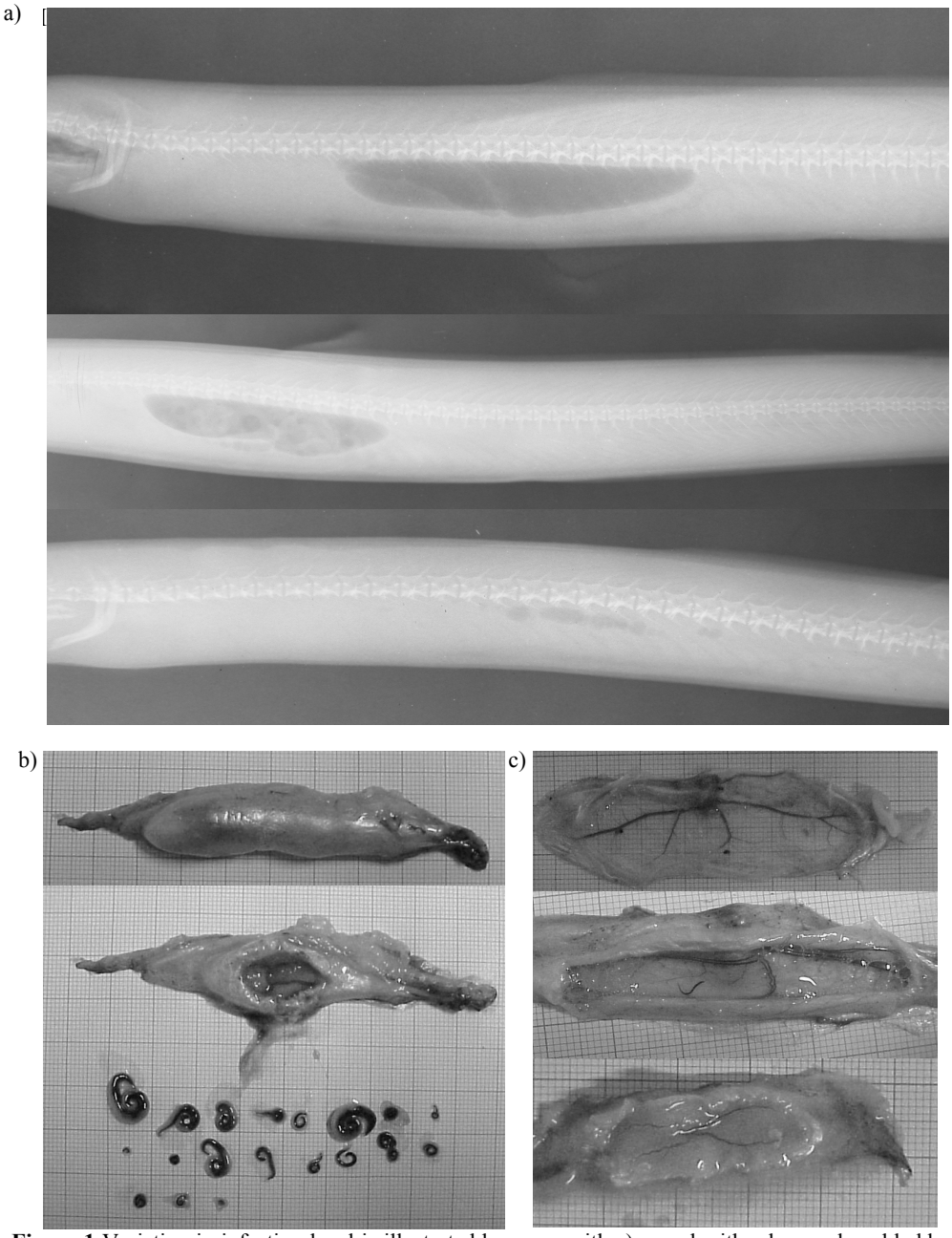


**Figure 1** Variation in infection level is illustrated by x-ray with a) an eel with a large, clean bladder (top), an eel with a medium sized bladder with visible parasites (middle) and an eel with a small bladder with minimal volume (bottom), b) Dissection of the swim-bladder, whole (top) and cut open (bottom) showing abundance of 19 parasites of various sizes and c) three levels of swim-bladder wall transparency, -thickness and length showing a large, thin-walled transparent swim-bladder (top), a medium-sized swim-bladder with a thicker wall (middle) and a small thick-walled non-transparent swim-bladder (bottom).

# Chapter 3

**Swim efficiency and reproductive migration of silver eels**

**are severely impaired by the swim-bladder parasite *Anguillicola crassus***

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CHAPTER 3

ABSTRACT

Infection with the swim-bladder parasite *Anguillicola crassus* is suggested as one of the causes of the collapse of eel populations worldwide. This nematode has been introduced 20 to 30 years ago from Asia and parasitised in a short time various eel species in different geographical regions of the world. The effects are energy drain due to its sanguivorous activities and mechanical damage of the swim-bladder wall by its migratory activity. These effects are hypothesized to impair the spawning migration of the European eel. In this study, we have investigated the effects of infection on swim performance. We hypothesized that parasitic sanguivorous activities reduce swim endurance while the mechanical damage impairs buoyancy control. Eighty eels suffering various degrees of infection have been introduced in the swim-tunnels and subjected to a swim fitness test. For the first time, oxygen consumption was measured of large infected silver eels swimming at different swim speeds, allowing to determine swim efficiencies. We found that especially silver eels are target of infection. Infected eels have lower cruise speeds and higher cost of transport. Eels that are not infected but contain a swim-bladder damaged by previous infection, show similar effects. Almost half of these eels stopped swimming at low speeds < 0.7 m/s. Effects thus seem to be associated with swim-bladder disfunction and the resulting loss of neutral buoyancy. This leads to the conclusion that infected eels with damaged swim-bladders will likely fail to reach the spawning grounds. Simulated migration trials confirmed fast migration failure (<1,000-km). This study shows that *A. crassus*-infection severely impairs the reproductive potential of eel. Recent studies indicated similar roles for PCB pollution and virus infection. We can therefore conclude that the downfall of quality of future genitors may well be a major acting force behind the eel's world-wide collapse.

## INTRODUCTION

Eel populations worldwide are dangerously close to collapse (Anonymous, 2003). Rapid decline started in the 80s and ever since no signs of recovery have been observed. Several causes have been suggested such as over fishing, habitat destruction, pollution and introduction of new diseases. Between 20 and 30 years ago two new diseases were introduced from Asia *e.g*. a virus EVEX (van Ginneken et al., 2004, 2005c) and a nematode infection with *Anguillicola crassus*, originally a parasite of the Japanese eel *A. japonica.* It took about one decade to spread the *Anguillicola crassus* infection over large parts of Europe (Neumann, 1985; Székely et al., 1991; Moravec, 1992; Evans & Matthews, 1999) and more recently it also reached the United States (Johnson et al., 1995). In a short time, various eel species in different geographical regions of the world were parasitised (Moravec & Taraschewski, 1988), likely due to worldwide eel shipments.

Since its introduction in Europe, many authors described its life cycle (Haenen et al., 1989; De Charleroy et al., 1990; Thomas, 1993). Adults of *A. crassus* reside in the swim-bladder. Eels are physostomes, which is considered a primitive condition and means that the swim-bladder has an open connection with the environment through the gut. The swim-bladder can be filled with $O_2$ from the gas gland or, with eel, also by gulping air (Bone et al., 1999). The main function of the swim-bladder is to obtain neutral buoyancy. In the lumen, the parasites feed on eel blood. Here the females produce eggs, which are

passively transported via the pneumatic duct to the oesophagus and finally through the digestive tract into the environment. The hatched larvae are eaten by copepodes that serve as intermediate hosts. The copepods are eaten by a number of fish species (Thomas & Ollevier, 1992; Székely, 1994; Pazooki & Székely, 1994) and other animals like aquatic insects, crustacea, snails and amphibians that serve as new paratenic hosts (Kennedy & Fitch, 1990; Thomas & Ollevier, 1992; Moravec & Konecny, 1994; Pazooki & Székely, 1994; Székely, 1995; Moravec, 1996; Moravec & Skorikova, 1998). All may serve as eel's prey. Larvae of *A. crassus* migrate directly through the wall of the digestive tract of the eel to the swim-bladder wall and finally end up in the lumen where they mature.

The life cycle of *A. crassus* in Japanese eel lasts for about one year (Egusa, 1979) while in European eel it takes only two months (De Charleroy et al., 1990). The infection causes lesions in European eel in contrast to infection of Japanese eel (Egusa, 1979). In addition, the number of parasites per infected eel is much higher in European eel (Egusa, 1979). Furthermore, parasites display higher survival rates and a higher reproductive success (Knopf & Mahnke, 2004). Clearly the European eel is more sensitive and less effective in its defense against *A. crassus*. Growth of infected eels was found to be reduced (Boon et al., 1990a), but mass mortality is thus far only observed in combination with additional unfavourable conditions, such as unusually long lasting high water temperature in Lake Balaton (Molnár et al., 1991; Molnár et al., 1993), and serious bacterial infections in Dutch eel farms (Van Banning and Haenen, 1990).

There are basically two kinds of adverse effects of *A. crassus* infection (Höglund et al., 1992): 1) energy drain due to sanguivorous activities of the parasite per se, and 2) mechanical damage of the swim-bladder wall. Concerning effect 1, Boon et al. (1990b) found that the sanguivorous activities of the parasites decrease the number of circulating erythrocytes and therefore the oxygen carrying capacity. Highly infected active eels are therefore presumed to have lower aerobic performance. Molnár (1993) proved that in decreasing oxygen content of the water severely infected eels die first,while uninfected specimens endure the hypoxic condition for a long time. Concerning effect 2, the migratory activity of the larvae in the swim bladder wall and the direct invasion of the pre-adults and adults in blood vessels result in extensive damage of the bladder wall (Molnár et al., 1993). Pathological changes include haemorrhages, formation of parasitic nodules, inflammatory cell proliferation, hypertrophy of connective tissue, necrotic areas and oedema. These changes eventually cause substantial thickening of the swim bladder wall (Molnár et al., 1993; Beregi et al., 1998) and shrinkage of the swim bladder.

Effects of severe *A. crassus* infection are hypothesized to impair the migration to the spawning grounds in the Sargasso Sea and therefore also impair reproduction. Since eels migrate about 5,500-km, probably at great depths, a decrease of oxygen carrying capacity and disfunctionality of the swim bladder will likely reduce the swimming capacity. Parasitism does not seem to impede pressure resistance (Vettier et al., 2003). However, eels rested during pressure exposure, so this experiment did not provide evidence for a functional swim-bladder. Two earlier studies investigated the influence of *A. crassus* on swimming of eel. Sprengel & Lüchtenberg (1991) found reduction of maximum swimming speed of eels between 17 and 45 cm. Heavy infected eels showed a reduction of maximally 18.6%, lowering average swim speeds from 0.725 to 0.590 m/s. However, in contrast, Münderle et al. (2004) could not verify those results for similar sized eels (40.3 ± 2.7 cm,

81 ± 16 g) at similar speeds of 0.62 m/s. Maximum swim speed, swimming performance and oxygen consumption were found similar between infected and uninfected eels. However, these studies were performed with small eels (maximum 45 cm) without specification of sex and life stage (silver males or yellow). Obviously large silver eels should be tested over long distance and periods, as not only swim speed but particularly a low cost of transport and a high endurance are crucial for long distance migration. Moreover, until now no discrimination was made between the direct and indirect effects of infection on swimming. When for instance a silver eel with a perfect condition and a functional swim-bladder is infected with many small larvae, then the initial migration phase of the eel would proceed without any problems. However, with increasing parasite load, more and more blood will be drained, resulting after some time in impaired swim performance. On the other hand, when an eel recovered from a severe infection, it would have a thickened swim-bladder wall. In that case the condition of the eel may be perfect, however, it would be unable to control buoyancy, and will thus still be incapable to reach the spawning site.

Recently, we developed an experimental test to quantify swim performance (*chapter 2*). This single-day 'swim fitness' test is an incremental speed test that can be used to predict endurance performance. The objective of this study is to investigate the relation between swim endurance and the adverse effects of *A. crassus* infection: 1) energy drain by parasites, and 2) buoyancy loss due to the mechanical damage of the swim-bladder wall.

## MATERIALS AND METHODS

*Choice of experimental eels*

For this study, we used eels from Lake Balaton because of two reasons. Firstly, the population of Lake Balaton eels generally displays high infection levels, especially at the end of the summer, which caused massive mortality in the past (Molnár et al., 1991, 1993). Secondly, Lake Balaton eels were at least 12 years old at the time of experiments since the lake was last restocked with glass eels in spring 1991 and has no endemic eel population (Bíró, 1992).

*Catch, selection and x-ray of experimental eels*

At the end of August of the subsequent years 2002 (n=40) and 2003 (n=40), eels were caught by electrofishing in Lake Balaton (Hungary) in the region of Keszthely and Tihany. Eels were transported to the laboratory in oxygen-filled plastic bags and then kept in concrete basins or plastic tanks with flow-through water until they were scanned by means of x-ray (Fig. 1) using the method described by Beregi et al (1998) and Székely et al. (2004, 2005). X-ray scans were used to measure the swim-bladder length (SBL) and to determine the actual swim-bladder status of the given eel specimen.. Eels were marked individually by injecting Passive Integral Transponder (PIT)-tags (TROVAN) subcutanously just behind the head. After a few days rest, the eels were packed into large oxygen-inflated nylon bags in boxes and sent to Leiden by air-mail early September (2002 and 2003).

*Swim-tunnel set-up and oxygen consumption*

A set of 22 Blazka-type 127-L swimtunnels as described by Van den Thillart et al. (2004) were used for the swim trials. The tunnels are placed in the direction of the Sargasso Sea (WNW) in a climatized room of about 100-$m^2$. The total water content of about 7000-L was recirculated continuously over a bio-filter. The illumination in the climatized room was switched to 670-nm light (bandwidth 20-nm). Based on pigment changes during silvering, it was assumed that this far-red light is invisible for eels (Pankhurst & Lythgoe, 1983). The oxygen level in each tunnel was measured continuously by an oxygen electrode (Mettler Toledo). The oxygen consumption rate was calculated from the oxygen decline after automatic closure of the water-inlet by a magnetic valve. From the decline of the $O_2$-concentration, the oxygen consumption rate was calculated following the formula:

$\dot{M}O_2 = 127 \cdot \Delta[O_2]/\Delta t$ (mg $O_2$/kg/h),

where: $\Delta[O_2]/\Delta t$ is the decrease of the oxygen content per hour.

*Experimental protocol*

Experiments were performed in 2002 and 2003. Eels (n=80) were introduced into the swim tunnels in fresh water at a constant temperature of 18 ± 1ºC at least two days before the experiment started. Before introduction, eels were anaesthetized with oil of cloves (1:10 dissolved in 100% ethanol using a dosage of 1-1.5 ml / l water). Oxygen electrodes were calibrated with sodium sulfite and air. Oxygen consumption was measured for a period of 3-4 h in rest. Eels were subjected to a swim fitness test described in *chapter 2*. In short: Eels started to swim at a swim speed ($U$) of 0.5 m/s for 2 h. During these 2 h, we measured the decreasing oxygen content in the tunnel for the first 1.5 h after which the tunnel was rinsed for 0.5 h. After these 2 h at 0.5 m/s, $U$ was raised with 0.1 m/s to 0.6 m/s for 2 h. Subsequently, this was repeated with steps of 0.1 m/s for $U$ up to 1.0 m/s. After each step the oxygen consumption was measured over the first 1.5 h, while during the last 0.5 h the water in the tunnel was refreshed. If oxygen levels came below 75% saturation, flushing occurred automatically raising AS level within 15 min to 85%. The swimming behaviour of the eels and their position in the swim tunnel was registered every 15 min. When the fish fatigued during the trials, the velocity was lowered immediately to 0.1 m/s. This velocity can be considered as resting state as eels had the choice either to swim or to rest.

*Swim parameters*

To characterise swim capacity and efficiency we derived five parameters (see *chapter 2*):

1) Oxygen consumption at rest ($\dot{M}O_{2\ rest}$) in mg $O_2$/h/kg,
2) The critical swim speed ($U_{crit}$) calculated according to Brett (1964),
3) Maximum $\dot{M}O_2$ at subcritical swim speeds ($\dot{M}O_{2\ max}$) in mg $O_2$/h/kg,
4) The speed at which the amount of work per distance reaches a minimum (Tucker, 1970): the optimum swim speed ($U_{opt}$),
5) The cost of transport (COT) at $U_{opt}$ in mg $O_2$/h/kg.

The $U_{opt}$ was determined by plotting a polynomial trendline through COT values vs. swim speeds per individual eel. The point on this trendline with the lowest COT was considered the $U_{opt}$ and was calculated by equaling the derative of the function of the polynomial

trendline to zero. By filling in this value again in the function of the polynomial trendline, the corresponding COT could be obtained.

*Measurements and sampling*

Morphometric parameters of the eels were measured before they were introduced into the swim tunnels including: bodylength (BL), bodyweight (BW), eye diameter horizontal (EDh) and vertical (EDv), pectoral fin length (PFL), BL (cm) and BW (g) and:

Fulton's condition factor $K= 100* BW/BL^3$.

The eye index according to Pankhurst (1982) $EI= 100* ((EDh+EDv)/4)^2\pi/10*BL)$

The pectoral fin index according to Durif et al. (2005) $PFI= 100* PF/BL$

The silver index according to Durif et al. (2005) based on BL, BW, ED and PF.

From the eels of 2003, 0.5 ml blood was taken before and after swimming. Haemotocrit (Hct) was determined immediately upon sampling. The remaining blood was centrifuged for 5 min at 14,000 rpm and bloodplasma was stored at -80˚C for later analysis of total blood protein (TP). Pre- and post swimming bloodplasma was defrosted on ice, 30* diluted and measured for TP content with a bicinchoninic acid protein assay reagent (assay #23225, Pierce Chemical Company, USA).

The swim-bladder was dissected and photographed on paper with a reference mm grid (Fig. 1). The bladder was cut open and the number of parasites was determined (Fig. 1). Parasites were preserved in 4% buffered formalin. These samples were used for wet weight determination of parasites (PW). For the determination of the direct effects of the infection i.e. the sanguivorous activities of the pre-adult and adult parasites, we calculated the weight of the parasites relative to the weight of the eel as parasite index (PI):

$PI = (PW / BW)$,

where PW is the parasite total weight (mg) and BW is the eel body weight (kg).

For determination of indirect effects of infection by mechanical damage of the swim-bladder wall, we calculated the length of the swim bladder relative to length of the eel as swim-bladder index (SBI):

$SBI = (SBL / BL)$,

where SBL is the swim-bladder length (cm) and BL is the eel body length (cm).

*Statistics*

Normality of data distribution was tested with Kolmogorov-Smirnov tests. For comparison of parameters before and after swimming one-tailed paired t-tests were performed. For comparison of parameters between swimmers and eels that fatigued at low speeds (drop-outs) and between eels of various silver stages, one-tailed unpaired t-tests were performed. For comparison of swim parameters between healthy, infected and damaged groups of eels, one-tailed univariate analyses of covariance (ANCOVA) was performed. Bodylength or -weight was used as cofactor. In case the cofactor did not have significant influence and to estimate between which groups the effect was significant, ANOVA with post-hoc Bonferoni correction was performed. Comparison of the number of eels that either stopped swimming before reaching a swim speed *U* of 0.7 m/s or continued swimming thereafter was tested with a Mann-Whitney U test. For correlation analyses, one-tailed Pearson tests were performed. All tests were performed in SPSS 10.0 for Windows. Results were calculated and plotted as means ± SD.

RESULTS

*Status of eels before swimming*

The experimental eels (n=80) measured 67 ± 6 cm (range 54-82 cm), weighed 466 ± 145 g (range 228-865 g), and had a condition factor K of 0.15 ± 0.02 (Table 1). The mean eye index (EI) was 8.38 ± 2.50; 71% of the eels had EI>6.5 and could thus be considered as silver. Among the experimental eels only 4% were in stage FII (residents), 56% in stage FIII (premigrants) and 40% in stage FV (active migrants), no eels were in stage FIV. The pectoral fin index (PFI) showed little variation between (yellow and silver) eels and was found 4.80 ± 0.44%. When considering characteristics of eels in the various migratory stages, stage FII residents (n=3) were the smallest at 57 ± 1 cm weighing 279 ± 12 g with a K of 0.15 ± 0.01. Stage FIII premigrants (n=39) were larger and measured 64 ± 5 cm weighing 390 ± 97 g with a K of 0.15 ± 0.01. Stage FV migrants (n=35) were the largest measuring 71 ± 5 cm weighing 573 ± 124 g with a K of 0.16 ± 0.02.

**Table 1** Morphometric parameters (mean ± SD) of experimental eels (BL= body length, BW= body weight, K= condition factor, EI= eye index, PFI= pectoral fin index, SI= silver stage).

| parameters | mean | SD | range |
|---|---|---|---|
| **BL (cm)** | 67 | 6 | 54-82 |
| **BW (g)** | 466 | 145 | 228-865 |
| **K** | 0.15 | 0.02 | 0.11-0.20 |
| **EI** | 8.38 | 2.50 | 4.99-16.34 |
| **PFI** | 4.80 | 0.44 | 3.80-6.05 |
| **SI** | 3.9 | 1.1 | 2,3,5 |

*Anguillicola crassus infection and swim-bladder damage*

The swim-bladder length (SBL) as indicator of shrinkage by damage was non-invasively determined by X-ray before swimming of 78 eels (Fig. 1a), two eels were not scanned. The SBL was 7.22 ± 2.45 cm (range 2.1-12 cm; Table 2). Relatively to the length of the eel (swim-bladder index SBI) these values were 10.8 ± 3.5%. After swimming, swim-bladders were dissected. The numbers of parasites were found between 0 up to 28 (Fig. 1b; Table 2). Parasite weight (PW) was between 0 up to 1.93 g. Relatively to the weight of the eel (parasite index PI) these values were 29.9 ± 51.2 mg/kg. We observed that the SBL was correlated to its volume, transparency, and thickness of the wall (Fig. 1c). Eels had swim-bladders with SBIs in the range 5.0-15.6 (Fig. 2). Swim-bladders with SBI<10 contained 25% of the accumulative parasite weight while those with SBI>10 contained 75% of the accumulative parasite weight. Thus, larger swim-bladders exhibited higher parasite loads. Accordingly, when the swim-bladder was smaller, parasite load became smaller. Non-infected swim-bladders were found of all sizes. Large swim-bladders had thin, semi-transparent walls and showed only slight signs of damage (thickening) or pre-infection. The smallest swim-bladders had damaged and thickened walls that had severely

reduced the swim-bladder volume. This condition reflected the reaction of the swim-bladder to high pre-infection loads making it unsuitable even for re-infection.

**Table 2** Parasite characteristics (mean ± SD and range; n=80). Swim-bladder damage was indicated by its length (SBL) and was determined by X-ray. Infection load was given by the number of parasites and parasite weight (PW) and was determined by dissection.

| | | | n | mean | SD | min | max |
|---|---|---|---|---|---|---|---|
| **damage** | **SBL** | **(cm)** | 78 | 7.22 | 2.45 | 2.1 | 12.0 |
| | **SBI** | **(%)** | 78 | 10.8 | 3.5 | 3.0 | 19.0 |
| **infection** | **parasites** | **n** | 78 | 3.4 | 5.3 | 0 | 28.0 |
| | **PW** | **(g)** | 71 | 0.15 | 0.29 | 0 | 1.93 |
| | **PI** | **(mg/kg)** | 71 | 29.9 | 51.2 | 0 | 295.0 |

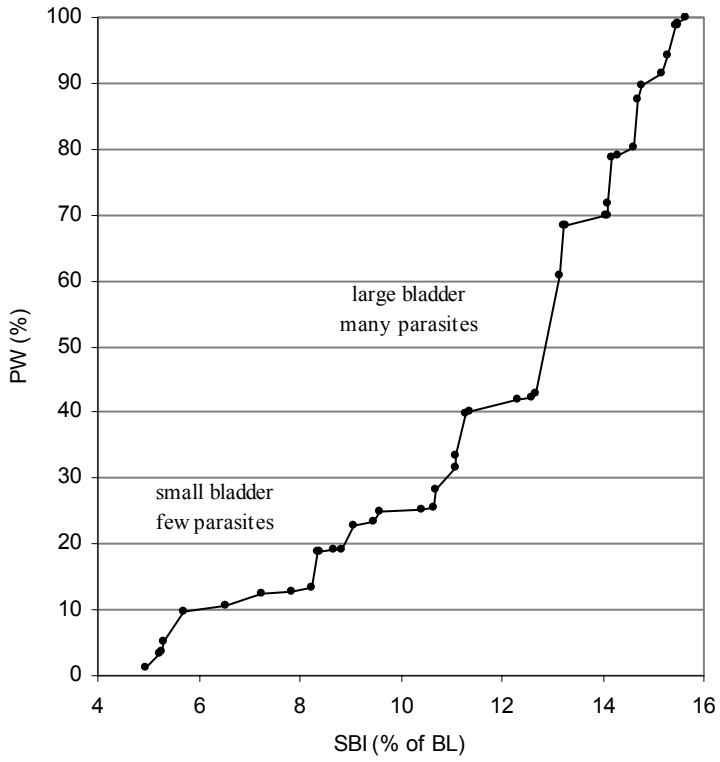


**Figure 2** Accumulative parasite weight PW (%) plot against the swim-bladder index SBI (% of BL) of infected eels. The smaller swim-bladders have lower parasite loads, while larger swim-bladders exhibit higher loads.

We pooled data in three groups based on the presence of parasites (infected/not infected) and, in the not infected eels, the SBI (larger or smaller than the mean): 1) a relatively healthy group represented by 13 eels with large swim-bladders (SBI ≥ 10) and

without parasites, 2) an infected group represented by 43 eels with all sized swim-bladders with parasites and 3) a damaged group represented by 14 eels with small swim-bladders (SBI < 10) and without parasites. All groups contained eels of similar BL and BW.

*Relation between silver stage, swim-bladder infection and damage*

Parasite index (PI) and Swim-bladder index (SBI) were compared between the eels of the represented silver stages (Fig. 3). A clear relation was found between the silver stage and the level of infection. The infection load significantly increased comparing the resident stage FII with the premigrant stage FIII ($P<0.001$) and the stage FII with the active migrants stage FV ($P<0.05$). This did not account for damage. Thus, silver migratory eels experienced highest infection levels (Fig. 3).

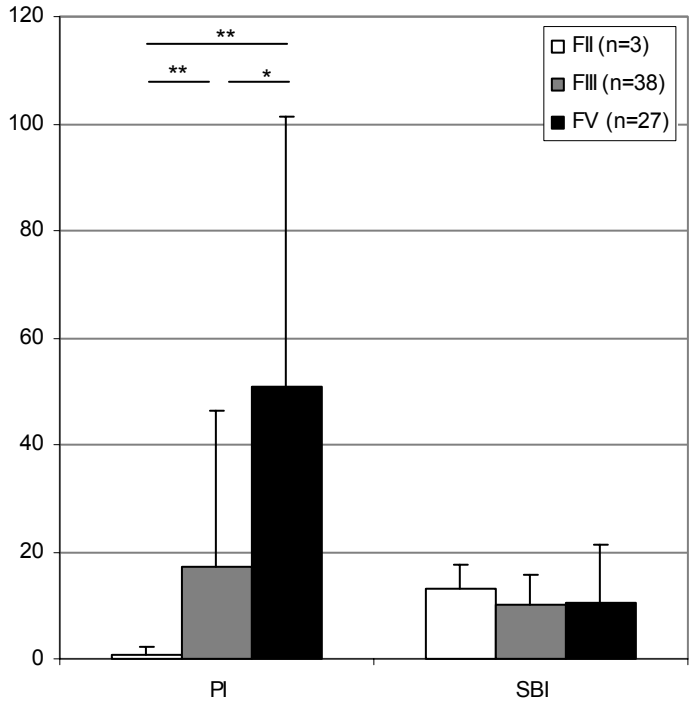


**Figure 3** Relative parasite weight (PI in mg/kg) and swim-bladder length (SBI in % of BL) in experimental eels representing silver stage FII, FIII and FV. Stage FIV was not represented. Active migrant silver eels harboured significantly more parasites and had a higher PI (* $P<0.05$ and ** $P<0.001$) than residents and pre-migrants. The SBI was not different between stages.

*Swimming of experimental eels*

Of 74 eels a complete set of swim data was collected. In general, two groups of swimming eels could be distinguished. A group of eels (from here on referred to as "drop-

outs", n=27) stopped swimming before reaching a swim speed *U* of 0.7 m/s. They swam unsteady and were not able to maintain balance in the swim-tunnel. The number of data points did not suffice to derive the polynomial and thus to determine the optimum swim speed ($U_{opt}$) and cost of transport (COT) for these eels. Another group consisted of steady swimmers (from here on referred to as "swimmers", n=47) that continued swimming at swim speeds ≥0.7 m/s. The drop-outs had a critical swim speed ($U_{crit}$) of 0.54 ± 0.07 m/s vs. 0.73 ± 0.09 m/s for the swimmers. Their $\dot{M}O_2$ rates were significantly higher. In rest, the difference between drop-outs and swimmers was not significant (resp. 41.7 ± 9.7 vs. 38.4 ± 8.0 mg/kg/h). But already at the start of swimming at 0.5 m/s the difference was significant (P<0.05) with resp. 129 ± 34 vs. 101 ± 30 mg/kg/h. The drop-outs showed indications of lower haematocrit Hct (P=0.07) and lower SBI (P=0.06).

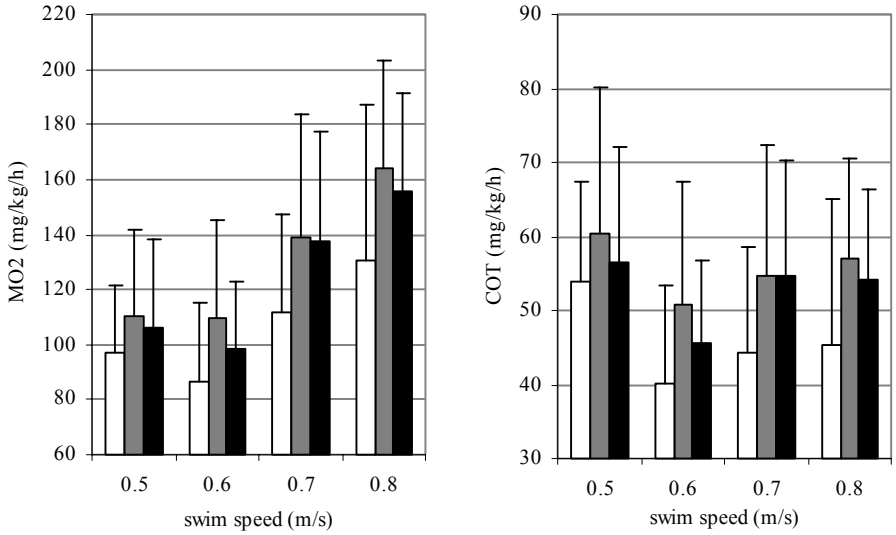


**Figure 4** Oxygen consumption levels ($\dot{M}O_2$) and cost of transport (COT) of healthy eels (white bars), infected eels (grey bars) and damaged eels (black bars) at swim speeds between 0.5 and 0.8 m/s. $\dot{M}O_2$ was higher (ANCOVA; P<0.01) for infected and damaged eels at all swim speeds. COT tended to be higher for infected and damaged eels at all swim speeds.

*Influence of infection and damage on swimming*

To analyse the influence of infection and damage on swimming, we compared values of swim parameters between the healthy, the infected and the damaged group by ANCOVA. Figure 4 shows oxygen consumption ($\dot{M}O_2$) and cost of transport (COT) levels at the various swim speeds. $\dot{M}O_2$ levels (P=0.01) and COT (ns) were found higher in the infected (13%) and damaged groups (9%) at all speeds. No difference was found in oxygen consumption in rest ($\dot{M}O_{2rest}$) but a significant effect of BW on $\dot{M}O_{2rest}$ (P<0.001) existed (Fig. 5a). Maximum oxygen consumption ($\dot{M}O_{2max}$) was higher in infected and damaged eels but not significantly (Fig. 5b). The effect of BW on $\dot{M}O_{2max}$ was significant (P<0.001).

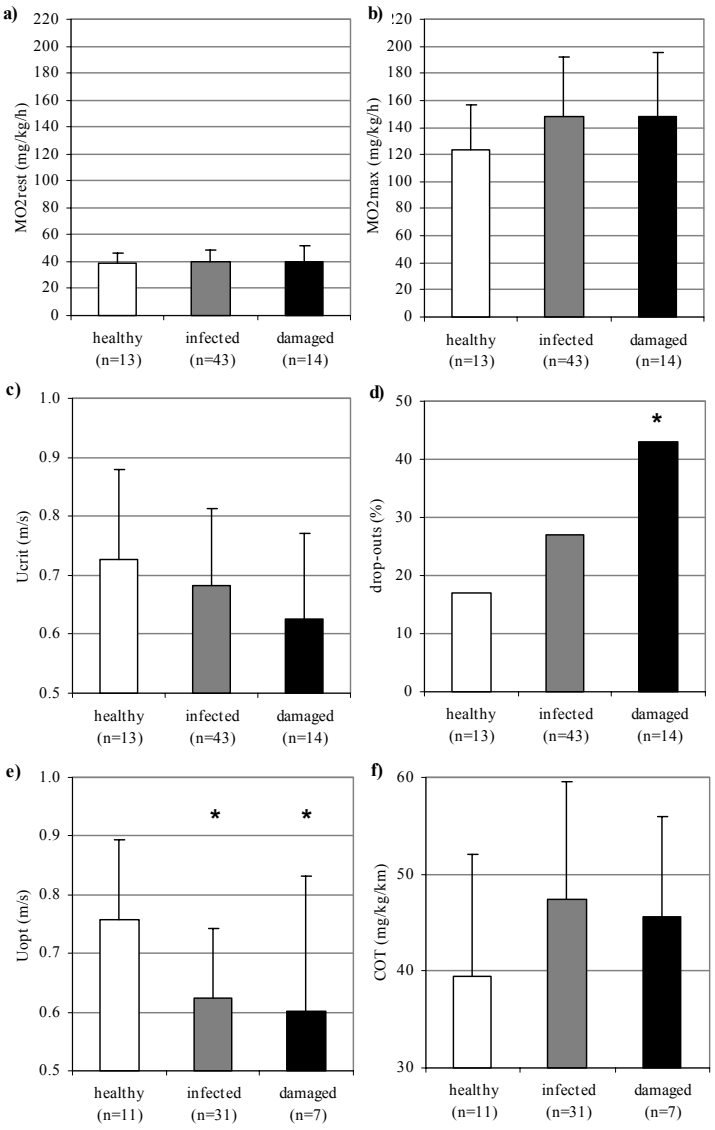


**Figure 5** Swim parameters of healthy eels (white bars), infected eels (grey bars) and damaged eels (black bars). Healthy eels had large swim-bladders (SBI ≥ 10) without parasites. Infected eels had all-sized swim-bladders with parasites. Damaged eels had small swim-bladders (SBI < 10) without parasites. Significant differences (P<0.05) are indicated by asteriks. No significant differences were found for a) oxygen consumption in rest ($\dot{M}O_{2\ rest}$) b) and maximal oxygen consumption ($\dot{M}O_{2\ max}$), c) critical swim speeds ($U_{crit}$) tended to decrease with increasing damage and d) 43% of these eels dropped out before reaching $U_{opt}$ (Mann-Whitney; P=0.03), e) eels with small swim-bladders had lower optimum swim speeds $U_{opt}$ (ANCOVA; P=0.01) and f) cost of transport (COT) tended to increase with increasing damage.

Critical swim speed ($U_{crit}$) was lower in the infected group and even more in the damaged group (Fig. 5c) but not significantly different. There was a significant effect of BL on $U_{crit}$ (P=0.01). In the healthy group of eels, the percentage of drop-outs was 17% (Fig. 5d). In the infected group of eels, this percentage was higher with 27%. Of the damaged eels, 43% dropped out which was significantly different (P<0.05) from the healthy eels (Fig. 5d). The optimum swim speed ($U_{opt}$) was 18 and 21% lower (P=0.01) in resp. infected eels and eels with damaged swim-bladders (Fig. 5e). Healthy eels had COT values of 40 mg/kg/h that increased 18 and 21% in respectively damaged eels and infected eels (Fig. 5f).

*Blood parameters before and after swimming*

The average haematocrit (Hct) percentage before swimming (eels 2003 only; n=40) was 31.4 ± 7.6 % (Table 3). Total protein (TP) content was 52.8 ± 5.6 mg/ml bloodplasma. No relation was found with silver stage (premigrants vs. migrants). Stage FIII premigrants had a Hct 30.2 ± 9.3% (n=18 eels from 2003). TP content was 53.6 ± 6.0 mg/ml bloodplasma. Stage FV migrants had Hct values of 32.4 ± 5.9% (n=22 eels from 2003). TP content was 52.1 ± 5.2 mg/ml bloodplasma. Blood parameters of stage FV migrants were similar as those of stage FIII eels, indicating that there was no difference in physical condition.

The average Hct percentage of 32.8 ± 5.7% after swimming was slightly higher than before but not significantly different (Table 3). The same applied to TP content with 53.5 ± 4.7 mg/ml bloodplasm. No correlations were found between the parasite index (PI) vs. Hct and TP. Although weak, decreasing trendlines of PI vs. Hct and TP could be plot, values for infected eels fell well within the large range of indivual variation of parasite-free eels. Correlations found between PI and the difference of Hct and TP were not found *i.e.* high parasite loads did not correlate with the level of change in Hct and TP due to swimming.

**Table 3** Blood characteristics before (pre) and after (post) swimming. Paired observations on eels from the 2003 experiment (n=40) of Hct and TP are shown. (SD= standard deviation, min= minimium and max= maximum).

| | | | n | mean | SD | min | max |
|---|---|---|---|---|---|---|---|
| **pre-swimming** | **Hct** | **(%)** | 40 | 31.4 | 7.6 | 10.2 | 48.4 |
| | **TP** | **(mg/ml)** | 40 | 52.8 | 5.6 | 38.3 | 64.6 |
| **post-swimming** | **Hct** | **(%)** | 40 | 32.8 | 5.7 | 20.6 | 44.4 |
| | **TP** | **(mg/ml)** | 40 | 53.5 | 4.7 | 46.9 | 64.3 |

## DISCUSSION

*Infection and damage*

This study attempted for the first time to estimate the effects of the *A. crassus* infection on swimming with respect to energy drain and swim-bladder damage. We used the relative parasite weight as a parameter for infection load and the swim-bladder length as parameter for the degree of damage. We observed that the length of the swim-bladder was correlated to its transparency and thickness of the wall (Palstra et al, unpublished data). The level of infection may impair the eel's condition and its endurance by energy drainage. The

level of damage may impair buoyancy control without affecting the eel's condition. High numbers of parasites (n>20) were found in large swim-bladders. Shorter swim-bladders contained less parasites. Density of parasites is constrained by space (Van Banning & Haenen, 1990; Ashworth & Kennedy, 1999; Lefebvre et al. 2002ab). Recently, Lefebvre & Crivelli (2004) showed that the infection rate is lower among eels with severely damaged swim-bladders. The shortest swim bladders did not harbor any parasites. Damage of these swim-bladders was so high that they were considered as totally disfunctional. It seems plausible that this eventually represents the endstage for all heavily infected eels. The space in the swim-bladder has become very limited by the thickened walls in the shortened swim-bladder reducing the change for survival and making reinfection very unlikely.

We did not find significant correlations between infection and haematocrit (Hct) and total protein (TP) content. Results in literature are contradictory. Boon et al. (1989) did not find a significant correlation with Hct but in a later publication he did find negative correlations with Hct and proteins (Boon et al., 1990b). Höglund et al. (1992) did not find a correlation with Hct but these authors did find a significant positive correlation between infection and total serum protein. Kelly et al. (2000) did not find significant correlations between infection and Hct, plasma glucose and many other physiological parameters. Parasites do not seem to cause anaemia (also Höglund et al., 1992) like with the EVEX virus (van Ginneken et al., 2004). Würtz et al. (1996) concluded that it does not seem that parasites show any sanguivorous activities but feed on surrounding tissue as can be concluded from their proteolytic enzymes (Polzer & Taraschewski, 1993). Thus, evidence about sanguivorous activities of the swim-bladder parasite and energy drainage in this way is still controversial.

*Effects on swimming: reduced cruising ability and efficiency*

In this study, for the first time oxygen consumption ($\dot{M}O_2$) was measured during swimming of large infected eels at various speeds. We found that infection and, even more, damage had serious effects on cruising ability and efficiency. Both infection and damage caused higher $\dot{M}O_2$ levels (resp. 13 and 9% ; P=0.02) at all swim speeds. Eels with damaged swim-bladders had a 21% decreased optimum swim speed ($U_{opt}$). These eels tended to raise cost of transport (COT) up to 18% (not significant). Almost half of these eels (43% ; P<0.05) dropped out below a swim speed of 0.7 m/s. Effects of infection and damage were similar but more pronounced in the latter. We hypothesize that additional energy is required to maintain neutral buoyancy. In the case that the swim-bladder's volume is reduced (by parasites and shortening of the swim-bladder), neutral buoyancy may become lost. Lift may be provided dynamicly by the pectorals to compensate for the loss of neutral buoyancy by a reduced swim-bladder volume (Bone et al., 1999). This mechanism is also illustrated by the fact that scombroid species, which do not have a swim bladder, must swim continuously with pectoral fins extended which produces a lift to overcome negative buoyancy. The 2 species with the fastest speeds necessary to counter negative buoyancy; skipjack (kawakawa) *Euthynnus affinis*, and Pacific bonito *Sarda chiliensis*, do not possess a swim-bladder (Beamish, 1978). To achieve lift by the pectoral fins, eels need to change to a more tilted position in the water column. Increase of swim-bladder damage would cause eels to swim harder to compensate. We found that maximum aerobic swim speeds tended to be negatively affected by infection and damage levels, but not

significantly. Also Münderle et al. (2004) concluded that $U_{crit}$ for smaller eels was unaffected. Maximum aerobic speeds up to 1.64 BL/s were found for Lake Balaton eels and were comparable to those found for different other groups of eels (*chapter 2*). We can conclude that infected and especially damaged swim-bladders are reduced in volume that may cause loss of neutral buoyancy. Swim efficiency in eels with damaged swim-bladders is significantly impaired as indicated by higher $\dot{M}O_2$ and COT levels and lower $U_{opt.}$. These eels stop swimming at a swim speed below 0.7 m/s.

*Migration failure*

Infected eels and eels with damaged swim-bladders experiencing impairment of swim efficiency are likely to fail migration. We did observe that all eels from Lake Balaton that stopped swimming during simulated migration trials had heavily damaged swim-bladders (Palstra et al., unpublished results); 80% of these eels stopped within 42 days and before reaching 1,000-km. This represented 30% of the total number of eels. Furthermore, in experiments where we hormonally stimulated infected silver eels from the Loire River (France; Palstra et al., unpublished results), we found that infection levels were still high after 6 months of captivity in salt water, while there was no chance of reinfection. This means that parasites survive longer or may have parasite larvae that develop such that swim-bladder damage progresses even under salt water conditions (also Kennedy & Fitch, 1990; Kirk et al., 2002ab). Székely et al. (2005) confirmed that during prolonged laboratory maintenance of *A. crassus* infected eels no improvement can be observed in the condition of the swimbladders.

*Migratory silver eels are targets for infection*

The old age of >12 years of the experimental eels was confirmed in another study by otolith analysis (Palstra et al., unpublished results). All eels in that study were between 13 and 21 years of age (n=20). Tátrai et al. (2003) examined 114 Lake Balaton eels (395-690 mm long and 112-760g weight) and confirmed the old age. Among the experimental eels were residents (FII) and premigrants (FIII), but also 40% active migrants (FV). It seems curious that migrant stage FIV was not represented. This might well be due to the absence of major differences in pectoral fin length, an important discriminator between stage 4 and 5 according to the PCA cluster analysis plots of Durif et al. (2005). The high percentage of active migratory Lake Balaton silver eels is in contrast with Bíró (1992), who stated that Lake Balaton eels never become silver and do not migrate. Accordingly also with Székely et al. (1991), Molnár et al. (1991, 1993), Békési et al. (1997), Nimeth et al., (2000), Sures et al. (2001) and Vettier et al. (2003) who stated that metamorphosis and migratory activity were impeded.

We found that the silver eels clearly displayed highest infection levels. We believe that a shift to higher quantity and quality (by e.g. piscivory) of food preceding silvering may be the proximate cause of higher infection chances and rates. The highest infection levels in Lake Balaton are found at the end of the summer at the time of silvering. Since especially migratory silver eels are targeted by infection, the impact of the adverse effects of infection is greater.

*Reproductive failure by swim-bladder parasite*

When results are extrapolated to the field, we can conclude that the damage of the swim-bladder wall caused by parasite infection with *A. crassus* very likely leads to a fast migration failure. In most European habitats 40 up to 90% of the eel population is infected (Sprengel & Lüchtenberg, 1991; Wurtz et al., 1998; Lefebvre et al., 2002ab; Audenaert et al, 2003; Dekker, 2004; Lefebvre & Crivelli, 2004). In this study we found that especially the migratory silver eels are heavily infected thus rising the impact of the effects. We found that effects concern a significant impairment of swim efficiency causing failure of long-term migration. Thus, for the first time strong evidence of *A. crassus* having major implications on recruitment is provided. Since the decline of European eel populations had already started when the swim bladder parasite was introduced, it might not have been the single cause of the decline. Devastating effects were also found for the virus EVEX on long term swimming (van Ginneken et al., 2005c) and contamination with PCBs on long term swimming (van Ginneken et al., to be submitted) and even more so on embryonic survival and development (Palstra et al., 2006). All these agents determine the spawner quality as a product of their habitat. Since recruitment is the product of quantity and quality of spawners, downfall of quality may well be a major acting force behind eel's worldwide collapse.

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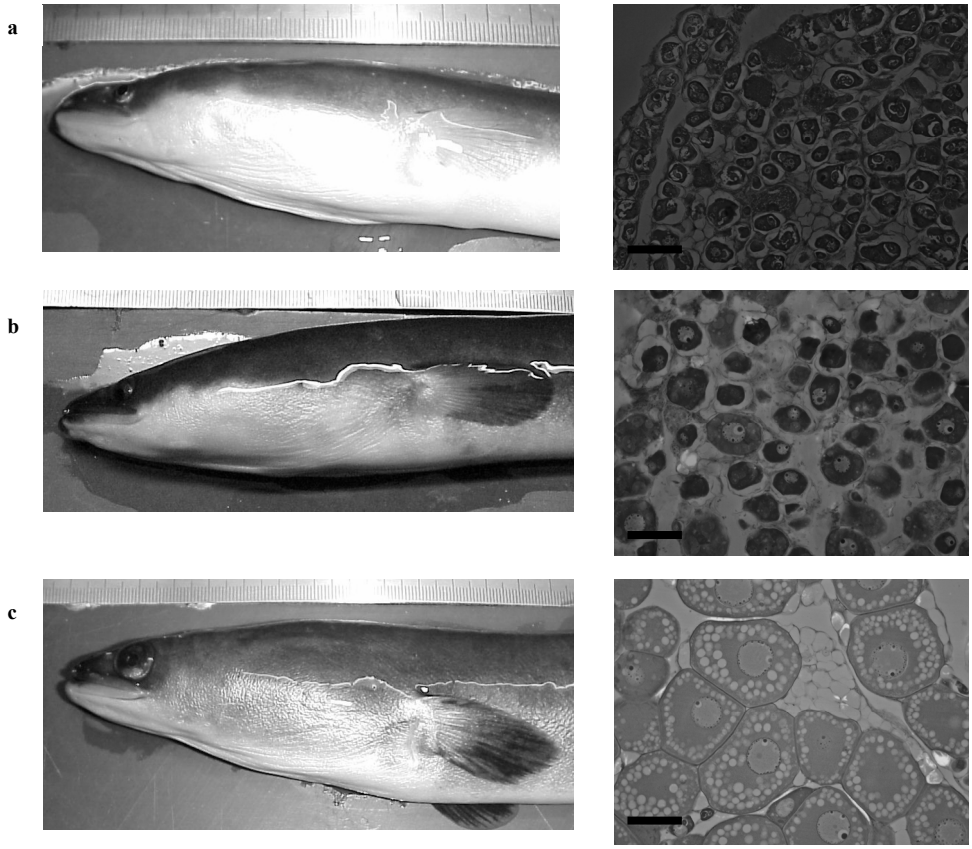


**Figure 2** Paired observations showing eye size and oocyte stage with a) an eel of 58 cm weighing 239 g with EI= 4.87, oocyte developmental stage 1-2, b) an eel of 67 cm weighing 424 g with EI= 9.14, oocyte stage 2-3 and c) an eel of 72 cm weighing 626 g with EI= 16.6, oocyte stage 3. Scale bars are 100 µm.

# Chapter 4

**Swimming stimulates oocyte development of European eel (*Anguilla anguilla*)**

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CHAPTER 4

ABSTRACT

European eel *Anguilla anguilla* is a primitive teleost with a semelparous life style and is one of the most extreme examples of reproductive homing. With this, eel is a perfect model to study the poorly understood relation between migration and maturation. Eels migrate downstream and leave the European coasts as silver eels in a prepubertal condition to arrive 4 to 5 months later in a mature condition at the spawning grounds in the Sargasso. As it is very likely that swimming triggers maturation during the 5,500-km migration, we hypothesized that swimming releases reproductive inhibition.

In this study, we subjected 55 old (>13 years) eels from Lake Balaton (Hungary) to swimming for durations of 1, 2 and 6 weeks. These eels were used since recent findings have suggested that older eels are more sensitive to mature. Changes in morphometry and oocyte development were determined to establish the silvering and maturation status. We found that swimming stimulates silvering, shown by enlargement of the eyes already within 2 weeks swimming. Furthermore, we found that swimming stimulates maturation. Already within 1 week swimming, the gonadal mass increased, oocytes became larger and large amounts of lipids were incorporated. However, vitellogenesis was not induced. Thus, it can be concluded that swimming plays a major role in release from reproductive inhibition and mobilisation of lipids to the oocytes. Findings support the hypothesis that older eels are more sensitive to mature.

INTRODUCTION

European eel spend their feeding stage as immature yellow eels in the fresh and brackish European waters. It appears that at the end of each growth season certain eels cease feeding and metamorphose (silvering) apprehending oceanic preparation. Probably the fat content is a key factor in the onset of migration (Larsson et al., 1990; Svedäng & Wickström, 1997). Drastic changes occur during silvering. Most apparent is the enlargement of the eyes which is used to discriminate between the yellow and silver phase by an index developed by Pankhurst (1982). Durif (et al., 2005) recently demonstrated that silvering and migration are closely related processes. As also the pectoral fins become longer (Durif et al., 2005), shape changes (Tesch, 2003). Durif et al. (2005) proposed an index on basis of length, weight, eye diameter and pectoral fin length, which provides an estimate of the proportion of silver eels that are true migrants. This was needed since their abundance was overestimated as demonstrated by Svedäng & Wickström (1997) and Feunteun et al. (2000).

Silver eels show an early development of the oocytes (reviewed for fish in general by Wallace & Selman, 1981 and Tyler & Sumpter, 1996, and for *A. japonica* by Adachi et al., 2003) and transform from oogonia to growing oocytes. After deposition of lipid droplets, further development includes vitellogenesis, maturation and ovulation which, for European eel, has only been observed when artificially matured through hormonal injections (Adachi et al., 2003, Palstra et al., 2005). Untreated silver eels are still at a prepubertal stage and far from sexual maturity (Larsen & Dufour, 1993; Dufour, 1994; Dufour et al., 2003) and will remain as such if prevented from oceanic migration to the spawning grounds in the Sargasso Sea (Dufour et al., 2003).

Prepubertal blockage of eel is due to a deficient GnRH stimulation and a simultaneous dopaminergic inhibition of the pituitary gonadotropes GTH-I (FSH-like) and GTH-II (LH-like). Severity of blockage is probably related to the migrational distance of the particular eel species (Todd, 1981). The question remains how and by what trigger or succeeding triggers eels are released from this blockage. Evidently, lipids play a major role in timing and thus triggering, since they are the ultimate requisite for successful reproduction. They are required for fuel as well as for incorporation in the oocytes (Palstra et al., 2005) thus forming a strong link between migration and maturation.

Studies on the interaction between migration and maturation are scarce which is surprising since especially migrant fish are often commercially interesting but difficult to reproduce in captivity. Exercise has never been thoroughly investigated as a stimulating factor of maturation of fish. Only recently, van Ginneken et al. (unpubl.) found increased oocyte diameters in 3 year old hatchery eels after swimming 5,500-km. Recently, we also found indications that older eels have higher capacity to incorporate fat from the muscle in the oocytes (Palstra et al, 2006). This finding was confirmed by Durif et al. (in press) who found positive correlations between age and condition factor, liver weight and vitellogenin. This implies that the advantage for eels having a greater age at reproduction results from higher energy stores and a more efficient vitellogenesis. Indeed, we found indications that older eels are more sensitive since they need less hormonal injections to mature (Palstra et al., unpubl.). We therefore hypothesize that older eels are more sensitive to induction of maturation.

In this study we investigated if eels are stimulated to silver and mature by swimming thereby releasing the prepubertal blockage. To exclude age as a limiting factor, we subjected eels from Lake Balaton (Hungary) to swim trials since these eels were at least 13 years old at the time of experimenting. Eels in Lake Balaton were stocked for the last time in 1990 and they cannot enter or escape since the lake is landlocked.

## MATERIALS & METHODS

### *Experimental animals*

At the end of August 2003 and at the end of September 2004, in total 120 eels were caught by electrofishery in Lake Balaton, Hungary, in the region of Keszthely and Tihany. They were transported to the laboratory in oxygen-filled foil bags and marked individually injecting PIT-TAGS (TROVAN) subcutanously just behind the head. Eels were then packed into large oxygen-inflated nylon bags in perspex and cardboard boxes after which they were sent to Leiden in early September (2003) or early October (2004).

### *Swim tunnels & conditions*

Swim experiments were performed in 22 Blazka-type callibrated swimtunnels described in detail by Van den Thillart et al. (2004). Swim tunnels were placed in the direction of the Sargasso Sea (WNW) in a climatized room of about 100-$m^2$. The total water content of about 7000-L was recirculated continuously over a bio-filter. The illumination in the climatized room was switched to 670-nm light (bandwidth 20-nm). Based on eye pigment changes during silvering, it was assumed that this far-red light is invisible for eels (Pankhurst & Lythgoe 1983). Indeed, the animals did not respond to

movements of the experimentor during red light illumination. Experiments were performed in air-saturated (> 75%) fresh water at 18±0.5ºC.

*Protocol experiment 1*

In experiment 1, 10 randomly chosen eels were anaesthetized, measured, sacrificed and sampled at arrival as control group. Forty eels in two groups of 20 were subjected to a swim fitness test that was in detail described in *chapter 2*. In short, eels were anaesthetized, measured and introduced into swim tunnels two days before the experiment started. Experimental eels swam for about 1 week consisting of 7 daily trials of maximally 12 hours each, depending on individual fatigue times. A group of 10 resting eels were kept during the experimental period in a 1500-l tank connected to a 2400-l recirculation system under dark conditions. PVC pipes were added to serve as shelter. At the end of the experiment, swimming and resting eels were anaesthetized, measured, sacrificed and sampled.

*Protocol experiment 2*

After arrival, 10 randomly chosen eels were anaesthetized, measured, sacrificed and sampled as control group. Fifteen randomly chosen eels were measured and introduced in the swim tunnels. They were allowed to swim at speeds of 0.5 BL/s. Resting eels were kept as described. After 2 weeks, 6 randomly chosen swimming eels were stopped, anaesthetized, measured, sacrificed and sampled as well as 10 resting eels. After 6 weeks, the remaining 9 eels were stopped, anaesthetized, measured, sacrificed and sampled as well as 6 remaining resting eels.

*Measurements & sampling*

Morphometric parameters included bodylength (BL), bodyweight (BW), eye diameters horizontal and vertical (EDh, EDv) and pectoral fin length (PFL). With these measurements we determined:

- Fulton's condition factor K using formula 1 (Table 1)
- The eye index according to Pankhurst (1982) EI using formula 2 (Table 1)
- The pectoral fin length index according to Durif et al. (2005) PFLI using formula 3 (Table 1)
- The silver index (SI) according to Durif et al. (2005) based on BL, BW, ED and PFL.

**Table 1** Formulas for sampling parameters.

1. $K= 100* BW/BL^3$
2. $EI= 100* ((EDh+EDv)/4)^2\pi/10*BL)$
3. $PFI= 100* PFL/BL$
4. Gonadosomatic index (GSI): (Weight gonads / Body weight) *100%
5. Digestive tract somatic index (DTSI): (Weight digestive tract / Body weight) *100%
6. Hepatosomatic index (HSI): (Weight liver / Body weight) *100%

Withdrawal of blood (500µl) was performed in the dorsal aorta in the tail with heparin flushed (10.000 IU/ml) 1 ml syringes which were immediately placed on ice. Hematocrit (Hct) values were measured in 9 µl whole blood samples in triplo using a micro-centrifuge

(Bayer, F.R.G.). Haemoglobin (Hb) content in 10 µl was determined in duplo by a spectrophotometer (LS50B, Perkin Elmer) measuring the absorbance at a fixed λ of 550 nm using the MPR 3 kit (1 ml, Roche Diagnostics GmbH). The MCHC (Mean Cellular Haemoglobin Content) was calculated dividing Hb by Hc. It then was centrifuged for 5 min at 14,000 rpm and bloodplasm was stored at -80˚C.

Liver, digestive tract and gonads were sampled and weighted. These were used to calculate the gonadosomatic index (GSI; Table 1, formula 4), digestive tract somatic index (DTSI; Table 1, formula 5) and the hepatosomatic index (HSI; Table 1, formula 6).
All eels were females. Samples were taken including gonads (portion of tissue from a standardised posterior location in Bouin solution stored at room temperature) and otolithes (sagitta) for age estimation.

*Otolithometry*

Age estimation was carried out in the laboratory of Cemagref, Bordeaux, France by otolithometry according to the method described by Daverat (2005a). After their extraction, otoliths were cleaned of all organic matter in distilled water, dried with ethanol, and then stored in eppendorf tubes. The otoliths were later embedded in synthetic resin (Synolithe), then polished to the nucleus with a polishing wheel (Streuers Rotopol-35) using 2 different grits of sandpaper (1200 and 2400). Fine polishing was done by hand with $Al_2O_3$ (1µm grain) on a polishing cloth. Etching was done using 10% EDTA. A drop of this solution was applied on the mold for a duration of 15 minutes. The otoliths were then rinsed with distilled water and stored in dry conditions. Yearly increments were revealed by staining with a drop of 5% Toluidine blue on the otolith and letting it dry. Growth rings were then counted under a microscope. The age of each eel was determined by the number of increments starting from the nucleus which was considered as year 1 of the eel's life.

*Histology*

To remove Bouin fixative the gonads were washed with 0.1M phosphate buffer for two days. Then the samples were put in a 70% alcohol solution and washed again for two days. To prepare them for embedding in Technovit 7100 (Kulzer Histo-Technik), the samples were taken through a series of accumulating alcohol percentage (70%, 80%, 90%, 96% and 100%, 1-1.5 h per step). Then they were stored in Technovit 7100 mix without the hardener for a few days to completely saturate them with the mixture (de Jonge et al., 2005). Then they were embedded in the plastic. Coupes of 10 µm thick were cut using the Leica microtome (Jung Polycut E). Three coupes were put on a slide and five slides per sample were made. Then they were dried on a heating plate. The slides were stained with Mayers Haematoxilin-Eosin for nuclei and cytoplasm. The oocytes were studied under the microscope (Nikon, Eclipse E400). For every oocyte a picture was taken (Nikon Coolpix 4500). Per coupe stage and diameter of ten oocytes was measured using UTHSCSA Image Tool 2.0 as well as the number and diameter of lipid droplets of stage 3 oocytes in experiment 2.

*Statistics*

Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov tests. Paired observations of morphometric parameters were tested

before and after swimming with student t-tests with one-tailed probabilities. For SI, a Wilcoxon test with one-tailed probabilities was used.

With a univariate general linear model (GLM), analysis of covariance (ANCOVA) with one-tailed probabilities was performed on log transformed unpaired observations in search for group effects in swim parameters with either BL (for PFL, PFW, ED) or BW (for GW, DTW, LW, Hct, Hb, MCHC, OD, number lipid droplets, diameter lipid droplets) as cofactors. ANCOVA was similarly performed for comparison between 2 weeks and 6 weeks swim groups. In case of occurrence of significant group effects, ANOVA with a post-hoc Bonferroni test was performed to specify the effects between particular groups. ANCOVA was especially required for the scalings difficulty between swim and rest groups in experiment 2 (Quinn & Keough, 2002).

Kruskal-Wallis tests with one-tailed probabilities were performed for comparison of SI and oocyte stage. Spearman correlation tests with one-tailed probabilities were performed between start parameters (BL*, BW*) vs. silvering parameters (EI*, SI*, HSI, DTSI) vs. maturation parameters (GSI, OS, OD) for control groups, but also for pre-swim groups for parameters marked with asterisks.

Pearson correlation tests with one-tailed probabilities were performed for comparing between paired swimming induced changes in EI after 2 and 6 wks swimming and for correlation between oocyte diameter and lipid droplet number and size. OD between stages was tested with student t-tests with one-tailed probabilities. All statistical tests were performed in SPSS 10.0 for Windows.

## RESULTS

*Silvering status before swimming*

Eels before swimming in experiment 1 were 69 ± 6 cm long, weighted 525 ± 142 g (Table 2a) and 70% was defined as silver eels (EI >6.5; Pankhurst, 1982). GSIs of eels in the control group were 0.59 ± 0.34 in a range 0.2-1. Eels before swimming 2 weeks in experiment 2 were 62 ± 4 cm long, weighted 347 ± 76 g (Table 2b) and 83% was defined as silver eels. Eels before swimming 6 weeks in experiment 2 were 63 ± 5 cm long, weighted 429 ± 137 g (Table 2b) and 33% was defined as silver eels. Eels in experiment 1 were larger and more silver. Experimental eels were assigned to SI stages 2, 3 and 5 (Durif et al., 2005). Not any fish was assigned to stage 4.

*Oocyte developmental status before swimming*

Before experiments, eels contained oocytes representing stages 1, 2 and 3 (Wallace & Selman, 1981). Stage 1 oocytes represented small oocytes organised as small oogonia in nests. They were oval shaped and the cytoplasm coloured dark. The nucleus was centred and round. It contained 1 to 3 larger nucleoli and about 20 smaller ones dispersed throughout the nucleus (Fig. 1a). Stage 2 oocytes represented larger oocytes with less dark coloured cytoplasm. In this phase a zona radiata was found starting to develop. The number of nucleoli had increased. Islands of lightly stained cytoplasm could be distinguished and connective tissue was present (Fig. 1b). Some stage 3 oocytes were found characterised by

**Table 2** Paired (pre-swim vs. post-swim) and unpaired (post-swim vs. rest, rest vs. control) measurements of a) experiment 1 (1 week swimming) and b) experiment 2 (2 and 6 weeks swimming). Expressed are number of countings n, the estimated age of swimmers (only experiment 2), morphometric parameters, blood parameters, internal parameters and oocyte parameters. Bold characters show significant differences (P<0.05) with * vs. pre-swim or control and ** vs. pre-swim or control and rest. Control eels were sacrificed at the start of experiments while resting eels were sacrificed at similar time points as swimming eels (1, 2 and 6 weeks).

a) experiment 1

| | | *1 week* | | |
|---|---|---|---|---|
| parameters | control | pre-swim | post-swim | rest |
| n | 10 | 40 | 40 | 9 |
| external | | | | |
| BL (cm) | 67±4 | 69±6 | 69±6 | 66±5 |
| BW (g) | 475±77 | 525±142 | **510±142*** | 437±123 |
| K | 0.16±0.01 | 0.16±0.02 | **0.15±0.02*** | 0.15±0.02 |
| PFLI | 4.68±0.39 | 4.96±0.43 | 4.88±0.42 | 4.68±0.49 |
| EI | 7.38±3.30 | 9.14±2.99 | 8.64±3.12 | 7.31±2.49 |
| SI | 3±1 | 4±1 | 4±1 | 3±1 |
| blood | | | | |
| Hct (%) | 33.3±6.2 | 31.4±7.6 | 32.8±5.7 | 33.5±7.3 |
| Hb (mM) | 6.26±1.38 | 5.95±1.69 | 5.77±1.00 | **5.09±1.22*** |
| MCHC (mM) | 19.1±4.1 | 18.9±2.9 | 17.8±3.2 | **15.3±2.3**** |
| internal | | | | |
| GSI | 0.59±0.34 | | 0.82±0.43 | 0.59±0.32 |
| DTSI | 2.42±0.65 | | **1.66±0.67*** | 2.01±0.52 |
| HSI | 0.92±0.09 | | 0.84±0.13 | 0.86±0.19 |
| oocytes | | | | |
| OS | 2.7±0.3 | | **2.8±0.4*** | **2.9±0.2*** |
| OD (µm) | 103±36 | | **155±47*** | 128±48 |

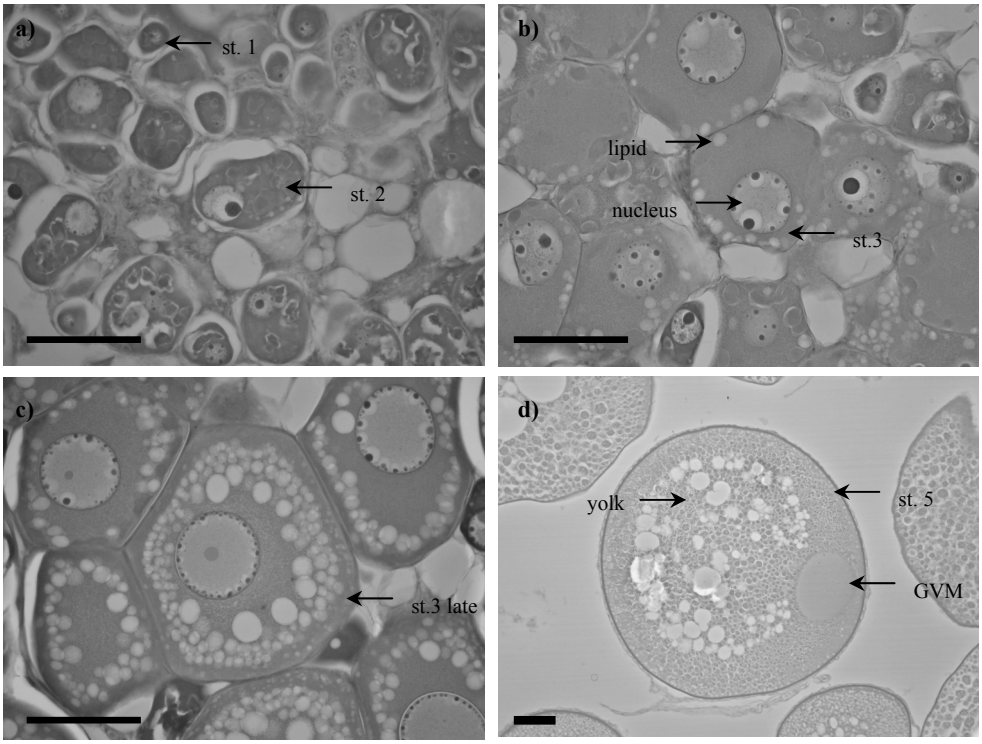
*** significant difference (P<0.05) vs. preswim / control**
**** significant difference (P<0.05) vs. preswim / control and rest**

b) experiment 2

| | | *2 weeks* | | | *6 weeks* | | |
|---|---|---|---|---|---|---|---|
| parameters | control | pre-swim | post-swim | rest | pre-swim | post-swim | rest |
| n | 10 | 6 | 6 | 10 | 9 | 9 | 6 |
| age (years) | | | 16±1 | | | 16±3 | |
| external | | | | | | | |
| BL (cm) | 59±5 | 62±4 | 62±4 | 55±2 | 63±5 | 63±5 | 53±2 |
| BW (g) | 267±68 | 347±76 | **333±63*** | 212±26 | 429±137 | **366±114*** | 174±23 |
| K | 0.13±0.01 | 0.14±0.02 | **0.14±0.01*** | 0.13±0.02 | 0.16±0.03 | **0.14±0.02*** | 0.12±0.02 |
| PFLI | 4.55±0.19 | 4.89±0.25 | 4.89±0.21 | 4.39±0.25 | 4.78±0.55 | 4.78±0.57 | 4.33±0.25 |
| PFWI | 2.32±0.26 | 2.64±0.43 | 2.45±0.46 | 2.30±0.17 | 2.52±0.18 | 2.52±0.16 | **2.03±0.21**** |
| EI | 6.20±1.81 | 8.32±2.20 | **10.31±2.84**** | 5.55±0.43 | 6.89±3.16 | **9.09±2.91**** | 5.53±0.60 |
| SI | 3±1 | 3±2 | 4±1 | 2±0 | 2±2 | **3±1** | 2±0 |
| blood | | | | | | | |
| Hct (%) | 27.8±8.0 | 23.1±9.3 | 24.8±5.1 | 27.4±5.9 | 27.5±6.2 | 30.7±5.9 | 32.7±4.23 |
| Hb (mM) | 7.34±3.10 | 6.80±2.53 | 5.17±0.40 | 5.38±0.94 | 7.24±1.05 | 6.31±1.62 | 6.65±1.00 |
| MCHC (mM) | 25.7±4.5 | 30.7±7.7 | **21.4±3.5*** | **20.1±3.0*** | 27.1±4.9 | **20.6±3.4*** | **20.3±1.7*** |
| internal | | | | | | | |
| GSI | 0.26±0.31 | | 0.74±0.48 | 0.13±0.12 | | **0.80±0.35**** | 0.38±0.18 |
| DTSI | 2.58±0.81 | | 1.95±0.77 | 2.40±0.52 | | 2.14±0.54 | **2.18±0.20**** |
| HSI | 1.13±0.20 | | 1.02±0.15 | **0.87±0.09**** | | 0.77±0.13 | **0.72±0.12**** |
| oocytes | | | | | | | |
| OS | 1.7±0.5 | | 2.4±0.7 | 1.5±0.2 | | **2.4±0.6**** | **1.7±0.1*** |
| OD (µm) | 83±39 | | 136±46 | 61±14 | | 109±41 | 94±17 |

*** significant difference (P<0.05) vs. preswim / control**
**** significant difference (P<0.05) vs. preswim / control and rest**

the presence of lipid droplets. In eels from control groups, stage 3 oocytes contained very few fat droplets, generally dispersed in the periphery of the ooplasm near the zona radiata (Fig. 1c). Mostly the nucleus was roundly shaped and nucleoli were found in the periphery of the nucleus. Less connective tissue was found surrounding the oocytes. Yolk globuli, characteristic for vitellogenesis (Fig. 1d), were not found.




**a**

**Figure 1** HE-staining of coupes of a) oocytes stage 1 and 2, b) oocytes early stage 3 with few lipid droplets and c) oocytes late stage 3 with a large number of fat droplets, d) for comparison: mature stage 5 oocytes displaying germinal vescicle migration (GVM) and with numerous yolk globuli of an artificially matured eel. Scale bars are 100 µm. Note that the mature stage 5 oocyte (d) is much larger than the oocytes in stage 1, 2 and 3 (a, b, c).

*Correlations size, silvering and oocyte developmental indicators*

Correlations between size, silvering and oocyte developmental indicators were analysed for experimental eels at the start (control and pre-swim measurements; Table 3). Significant positive correlations were found between size and silvering indicators and between size and maturation indicators. Between silvering and maturation indicators, significant positive correlations were found only between EI, SI and oocyte stage (Table 3; Fig. 2). Positive correlation with GSI was just not significant. No significant correlation was found between external indicators of the level of silvering EI and SI vs. DTSI and HSI.

**Table 3** Correlations and significance between size, silvering and oocyte developmental indicators. In bold are given significant correlations (P<0.05).

| | | BW | EI | SI | DTSI | HSI | GSI | OS | OD |
|---|---|---|---|---|---|---|---|---|---|
| **BL** | corr. | **0.944** | **0.740** | **0.680** | -0.218 | **0.478** | **0.703** | **0.900** | **0.612** |
| | P | **0.000** | **0.000** | **0.000** | 0.178 | **0.017** | **0.000** | **0.000** | **0.003** |
| | n | **75** | **75** | **75** | 20 | **20** | **20** | **19** | **19** |
| **BW** | corr. | | **0.729** | **0.658** | -0.147 | **-0.522** | **0.734** | **0.884** | **0.582** |
| | P | | **0.000** | **0.000** | 0.268 | **0.009** | **0.000** | **0.000** | **0.004** |
| | n | | **75** | **75** | 20 | **20** | **20** | **19** | **19** |
| **EI** | corr. | | | **0.884** | -0.211 | -0.062 | 0.311 | **0.510** | 0.212 |
| | P | | | **0.000** | 0.186 | 0.398 | 0.091 | **0.013** | 0.191 |
| | n | | | **75** | 20 | 20 | 20 | **19** | 19 |
| **SI** | corr. | | | | -0.090 | -0.020 | 0.351 | **0.603** | 0.269 |
| | P | | | | 0.353 | 0.467 | 0.065 | **0.003** | 0.133 |
| | n | | | | 20 | 20 | 20 | **19** | 19 |
| **DTSI** | corr. | | | | | -0.013 | **-0.432** | -0.323 | -0.203 |
| | P | | | | | 0.479 | **0.028** | 0.088 | 0.203 |
| | n | | | | | 20 | **20** | 19 | 19 |
| **HSI** | corr. | | | | | | **-0.342** | **-0.390** | -0.229 |
| | P | | | | | | **0.070** | **0.049** | 0.173 |
| | n | | | | | | **20** | **19** | 19 |
| **GSI** | corr. | | | | | | | **0.721** | **0.730** |
| | P | | | | | | | **0.000** | **0.000** |
| | n | | | | | | | **19** | **19** |
| **OS** | corr. | | | | | | | | **0.744** |
| | P | | | | | | | | **0.000** |
| | n | | | | | | | | **19** |

*Changes in silvering after 1 week swimming (experiment 1)*

Paired observations showed that after 1 week swimming, experimental eels showed a significant weight loss of 15 g (Table 2a). Also the K was significantly lower. No increase was observed of silvering indicators. Paired observations of blood characteristics showed no changes in Hct, Hb and MCHC in the swim group (Table 2a). The rest group showed a significant lower Hb and MCHC. The GSI of eels that had swum was on average 39% higher than control and rest groups but individual variation remained high and this difference was not significant (Table 2a). Percentages of eels with GSIs >1 were 11% in the rest group and 33% in the swim group. The DTSI was significantly lower in the swim group. HSI was lower in the rest and swim group but not significantly.

*Changes in silvering after 2 and 6 weeks swimming (experiment 2)*

Paired observations showed that after 2 weeks of swimming, experimental eels showed a significant weight loss of 14 g (Table 2b). Also the K was significantly lower. PFLI and PFWI did not show changes. The EI increased significantly from 8.32 ± 2.20 to 10.31 ± 2.84, also in comparison with the rest group (Fig. 3a). After 2 weeks, all eels showed increase of the EI of 0.83 up to 3.36 which is between 11 to 41% (Fig. 3a). Accordingly, this caused a rise in SI. Paired observations of blood characteristics showed a slight, not significant increase in Hct and a slight, not significant decrease in Hb (Table 2b). The resulting MCHC became significantly lower which was also observed in the rest group. The GSI was found on average 3-5 times higher though not significantly (Table 2b). The

number of eels with a GSI >1 was 50% in the swim group and significantly different from the control and rest group, as there were none in those groups. DTSI and HSI was lower but not significantly. HSI was found significantly lower in the rest group both vs control and swim group.

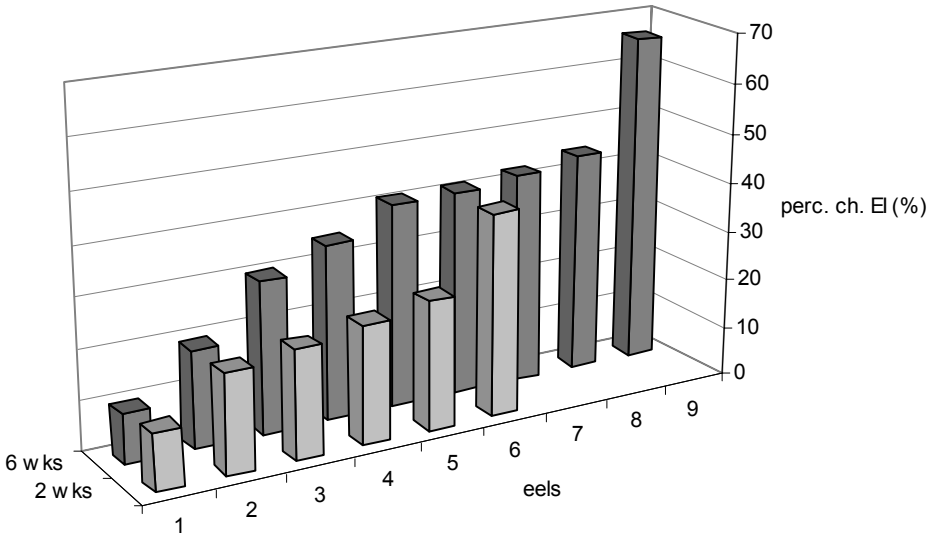



**Figure 3** Paired (pre vs. post-swim comparison of the same eels) individual percentual changes in EI after 2 and 6 wks swimming. All eels show increase after swimming. Changes are more pronounced after 6 weeks swimming.

Paired observations showed that after 6 weeks of swimming, experimental eels showed a significant weight loss of 63 g (Table 2). Again the K was significantly lower. PFLI and PFWI did not show changes in the swim group. The PFWI was however smaller in the rest group. The EI increased in the swim group significantly with 1.40 up to 3.50 which is between 10 up to 66% (Fig. 3b) creating a rise in the percentage of silver eels up to a 100%. This resulted in a significant change in the SI. Paired observations of blood characteristics showed the same changes as after 2 weeks swimming resulting in significant decrease of MCHC in the swim but also in the rest group (Table 2b). The GSI in the swim group was found significantly higher than in the control and rest group. Also in comparison with 2 weeks swimming, the GSI was significantly higher both in the swim group and rest group. DTSI and HSI were lower in the swim group but only significantly in the rest group. Also in comparison with 2 weeks swimming HSI was significantly lower (Table 2b).

*Changes in oocyte development after 1 week swimming (experiment 1)*

Eels of the control group in experiment 1 showed oocytes in stage 1, 2 and 3 with the latter oocytes just having entered this stage containing just a few lipid droplets. Eels showed heterogenous ovaria mainly with stage 2 and 3 oocytes (Fig. 4a). The average diameter of these oocytes was 103±36 μm. After 1 week, eels from both the swim group and rest group showed significant change in the average oocyte stage (Table 2a). Eels in the

rest group had mainly oocytes with very few lipid droplets. For 85% of the eels in the swim group, ovaria had stage 3 oocytes with much more lipid droplets. The oocyte diameter was higher in the rest group (128±48 μm) but only significant in the swim group (155±47 μm).

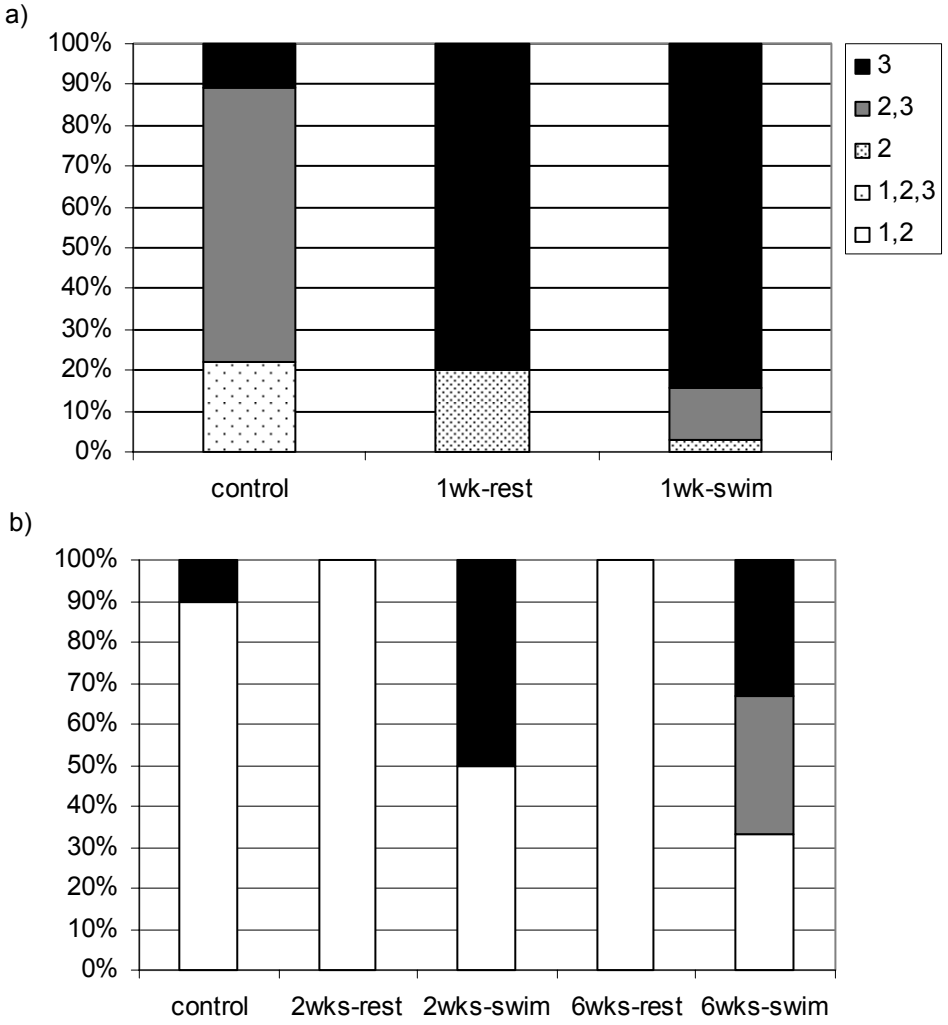



**Figure 4** Distribution of oocyte stages 1-3 in eels of control, rest and swim groups a) after 1 week and b) after 2 weeks and 6 weeks. Oocyte categories (legend) represent combinations of oocyte stages represented in individual eels. Five categories were found: eels contained oocytes in stage 1 and 2, in all stages, only in stage 2, in stage 2 and 3 or only in stage 3 oocytes.

*Changes in oocyte development after 2 and 6 weeks swimming (experiment 2)*

Eels of the controlgroup in experiment 2 showed oocytes in stage 1 and 2 except for one eel that showed only stage 3 oocytes, again with few lipid droplets. The average diameter of these oocytes was 83±39 µm. After 2 weeks of swimming, eels showed oocyte development. Changes in average stage and oocyte diameter were just not significant (Table 2b). However, 50% of the eels had solely stage 3 oocytes in the ovaria with large numbers of lipid droplets in contrast to eels in control and rest groups which only had stage 1 and 2 oocytes (Fig. 4b). After 6 weeks, eels from the swim group showed further oocyte development. The change of average oocyte stage in the swim group was significant vs. control and rest group (Table 2b). In the swim group, oocytes were on average larger (not significantly). In the rest group, eels had only oocytes representing stage 1 and 2. In the swim group, only 3 eels showed stage 1-2 oocytes. The other 6 showed stage 2-3 or 3 oocytes with large numbers of fat droplets (Fig. 4b). The change in the rest group was also significantly different from the control group. Although values were similar, the eels in the 6 weeks rest group were smaller. Average stage and diameter were significantly higher after 6 weeks resting than after 2 weeks resting (Table 2b).

Large variation in number and size of lipid droplets in stage 3 oocytes was observed. Fig. 5 shows that both number and size of lipid droplets were positively correlated (for both $P<0.01$) to the size of the oocytes (pooled samples) indicating that lipids had been incorporated. Stage 1 oocytes were on average 56 ± 14 µm (n=220). Stage 2 oocytes were significantly larger ($P<0.0001$) and on average 87 ± 23 µm (n=395). Stage 3 oocytes were again significantly larger ($P<0.0001$) and on average 159 ± 36 µm (n=165). They contained on average 45 ± 30 lipid droplets in a range between 6 and 102 which measured on average 11 ± 2 µm in a range between 5 and 17.

## DISCUSSION

*Swimming triggers silvering*

Swim exercise triggers increase of the eye index (EI) in all experimental eels already after 2 weeks of swimming. The EI was not increased in rest groups indicating that no time and/or starvation effect occurred. Swimming induced changes seemed even more apparant after 6 weeks swimming.

Already at the start, silver eels of the migrant SI stage 5 were present. They had GSIs >1 and most had already some stage 3 oocytes but only with few lipid droplets. The presence of active migratory Lake Balaton silver eels is in contrast with Bíró (1992), who stated that Lake Balaton eels never become silver. Surprisingly, the migrant SI stage 4 (Durif et al., 2005) was not represented at all, before and after experiments. No changes of the pectoral fins were observed due to swimming. This does not agree with the findings that pectoral fins become larger and change shape during silvering (Tesch, 2003), changes that are supposed to correlate with migration (Durif et al., 2005).

DTSI and HSI were found not to be correlated with silvering level but with maturation level. Therefore, it does not seem surprising that decreases in DTSI and HSI also appeared as a starvation effect. They were found equally lower in all swim and rest groups and thus without additional effects of swimming.

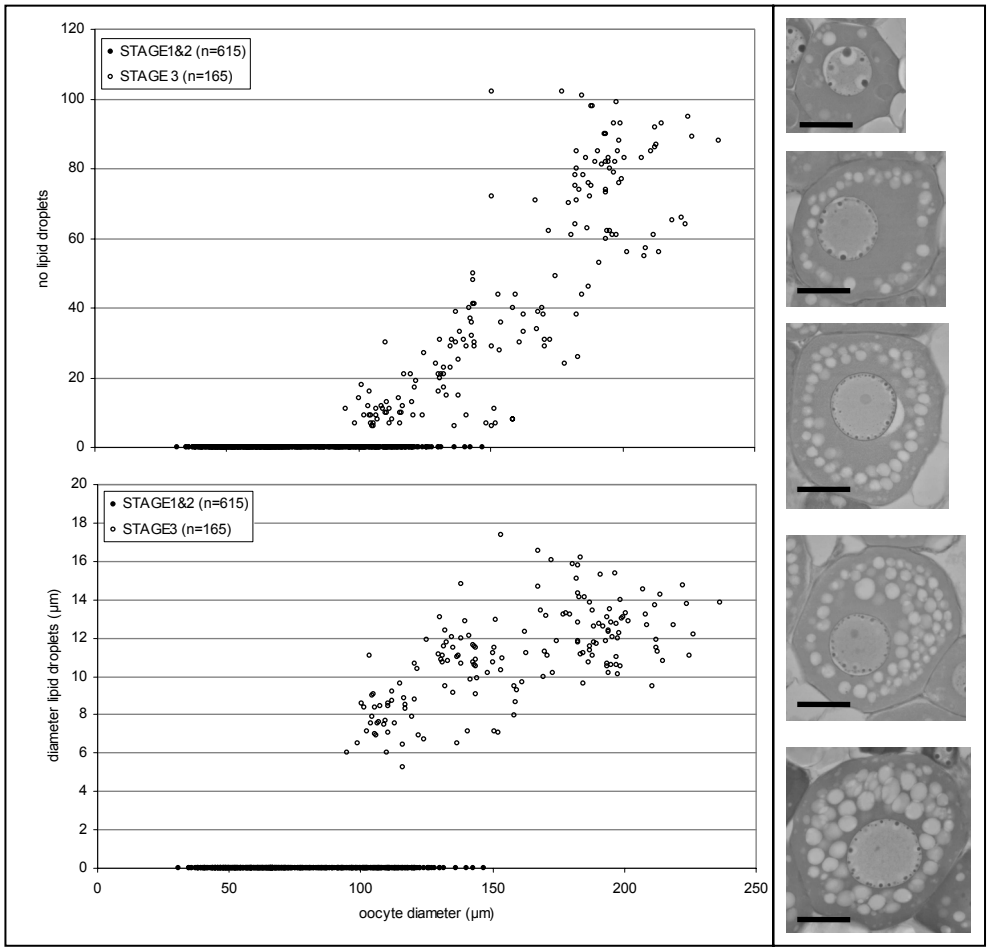



**Figure 5** Pooled oocyte samples of eels of experiment 2. Only swimming eels had stage 3 oocytes. A positive correlation existed for these oocytes between oocyte diameter and a) number of lipid droplets and b) diameter of the lipid droplets. The found diversity of number and size of fat droplets is illustrated by the oocytes in the pictures on the right. Oocytes have more and larger lipid droplets from top to bottom. Scale bars are 50 µm.

*Swimming triggers oocyte development*

Swim exercise triggers oocyte development. Significant increases were found in GSI, OS and OD in swimming eels. Swimming appears as a strong additional effect since also resting eels showed some development. After 6 weeks of swimming, changes were much more pronounced than after 2 weeks of swimming. GSI and oocyte diameter were significantly higher. Therefore, we can conclude that swim exercise not only triggers but also continues to stimulate oocyte development. We found that swimming induced shifting in oocyte development up to the lipid droplet stage that was characterised by high variation

in numbers of lipid droplets. Developments occured already after 1 week swimming and lasted up to swimming for 6 weeks. In this study we found that swimming caused a shift from stage 1-2 oocytes to stage 3 oocytes. Oocyte development also occured to a smaller extent in rest groups. Oocyte stage was found significantly higher in rest groups already after 1 week. GSI and oocyte diameters were significantly higher in the 6 weeks rest group in comparison with the 2 weeks rest group.
Recently, van Ginneken et al. (unpublished) simulated a complete migration of 5,500-km for 3 year old hatchery eels. These eels were 71±4 cm long and weighed 792±104 g, larger than the experimental eels in this study. Significant changes were found in pituitary GTH-II and plasma estradiol compared to the controls. The oocyte diameter was the only change that was a significant effect of swimmers compared to controls and resting eels. Unlike this study, van Ginneken et al. (unpublished) did not find differences in EI and GSI. The more explicit changes in this study, already after 2 weeks swimming, might be explained by the difference in age. The hatchery eels were only 3 years old while the Lake Balaton eels in our study were between 13 to 21 years old. This finding supports our hypothesis that older eels are more sensitive for maturation. It may well be that the eels in this study, prevented from migration in their natural habitat, are more sensitive since they have regressed from silver to yellow, maybe even multiple times during successive years (Durif et al., 2005). The different results suggest that age might be a key factor for the start of migration, for silvering, for the onset of maturation and reproductive success at large.

*Synchronous development of oocytes*

A point of discussion in literature concerns the question whether eels exhibit synchronous or asynchronous oocyte development. Eels are considered to have synchronous ovaries typical for semelparous teleosts (Wallace and Selman, 1981). When artificially matured, eels show however asynchronous development of oocytes maturing over several generations as shown by Palstra et al. (2005). In that study it is hypothesized that asynchronous development had an artificial rather than a natural origin. Oocytes were found to develop rather synchronized in this study, at least up to stage 3 (Fig. 4).

*Swimming does not trigger vitellogenesis*

In this study, we observed swimming induced oocyte development only up to stage 3: the lipid droplet stage. Stage 3 was found to be highly variable with respect to the arrangement, number and size of the lipid droplets. Oocytes were found with only few small droplets but especially oocytes of eels that had swum contained more than 100 larger droplets. This indicates that swimming induces the incorporation of fats. However, oocytes did not develop further than stage 3. 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), clear stage 3 previttelogenic oocytes (Colombo et al., 1984). This should indicate the start of vitellogenesis in *A. rostrata* according to Cottril et al. (2001). However, we did not observe any yolk globuli in the appropiate oocytes. In addition, the oocytes did not reach sizes that are characteristic for vitellogenesis. Vitellogenesis is the major cause for oocyte growth in teleosts in general (Tyler, 1991), including eel (Nagahama, 1994). Cottril et al. (2001) found oocytes of 200 µm for *A. rostrata* and considered them vittelogenic. Such values do, however, not correspond to the

growth typical for vitellogenesis. Adachi et al. (2003) stated for *A. japonica* that vitellogenesis begins when oocytes are about 250 µm in diameter. In this study we found maximum oocyte diameters of 236 µm. Also wild (untreated) migratory Loire eels did not contain vitellogenic oocytes (no yolk globuli, < 250 µm; Palstra et al., unpublished data). Recently, Vtg in the blood plasma of all the experimental eels of this study was determined by ELISA following the method of Burzawa-Gerard et al. (1991) by the group of Sylvie Dufour (MNHN, Paris). Vtg content in all samples was under the detection limit of 0.5 µg/ml. From this, we can conclude that already Vtg synthesis and secretion by the liver, hepatic vitelogenesis, is not triggered; a process which is under major control of E2. In general, Vtg levels remain very low for wild migratory silver eels. Van Ginneken & Dufour (unpublished) measured Vtg in 104 large female silver eels from Lake Grevelingen (The Netherlands), caught at the sluices at the Northsea side at 32 ppt about to start their oceanic migration. Of these eels, 96% showed similar low Vtg levels <0.5 µg/ml. Thus, a similar situation is reflected in the wild. Also Lokman et al. (2003) reported on basis of unpublished data (Lokman, Okumura, Adachi, Yamauchi) that Vtg synthesis as evidenced by Northern blot analysis of hepatic RNA, is not apparent in silver female *A. anguilla*.

*Triggering vitellogenesis*

We suppose that an extended swim period or another trigger is required for induction of vitellogenesis. Vitellogenesis might be induced only near or after arrival at the spawning grounds in the Sargasso. Since hepatic vitellogenesis is not induced by swimming, at least not within a swim period of 6 weeks, estradiol (E2) synthesis and release may thus be triggered but apparently not enough to induce vitellogenesis. Testosterone (T) synthesis and release, in contrast, is probably triggered by swimming resulting in the increase of the eye index. Recently, van Ginneken et al. (unpublished) found trends of increased 11-ketotestosterone and estradiol in the blood plasma of 3 year old hatchery eels after swimming 5,500-km. The reproductive axis in fish is closely related to fat metabolism since polyunsaturated fatty acids and their metabolites can have different modulatory effects on steroid metabolism and function (reviewed by Sorbera et al., 2001). Mobilisation of fats by fasting and, even more pronounced, by swimming may induce activation of the steroid metabolism. In the near future we will quantify the sex steroid levels in the blood plasma as well as expression of LH and FSH in the pituitary, expression of their receptors in the gonads and expression of vitelogenin 1 and 2, and estradiol receptors in the liver, to specify the mechanism behind the stimulation of maturation by swimming.

Thus, an extended swim period or some other factor is required to trigger the responsiveness of the liver to estrogen in the production of vittelogenins. Growth of oocytes is most pronounced during vitellogenesis and subsequent maturation. OD will increase 2-fold up to 400 µm during vitellogenesis and again 2-fold up to 800 µm due to hydration during final maturation (Palstra et al., 2005). These increases result in similar increases of gonadal mass and with that of the body diameter. This then causes increase of drag during swimming and with that increase of cost of transport. This situation is undesirable during migration.

*Future studies*

It is peculiar that the influence of swim exercise on maturation has never been thoroughly investigated, especially since migrant fish like tuna, salmon and eel are of major commercial interest but very difficult or even impossible to reproduce in captivity. Very recently, Patterson et al. (2004) recognized this issue also for salmon and stated that '*exercise associated with migration is presented as a potential obstacle to successful reproduction*' but that '*there has been no attempt…to reverse this paradigm and examine exercise as an integral part of normal reproductive development for long distance migrators*'. 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. In our study we found that swimming induces maturation of eel. Eel could serve as a model to elucidate the mechanism. Swim exercise may well be successfully included in future protocols for reproduction of eel in captivity. These are topics of our current investigations.

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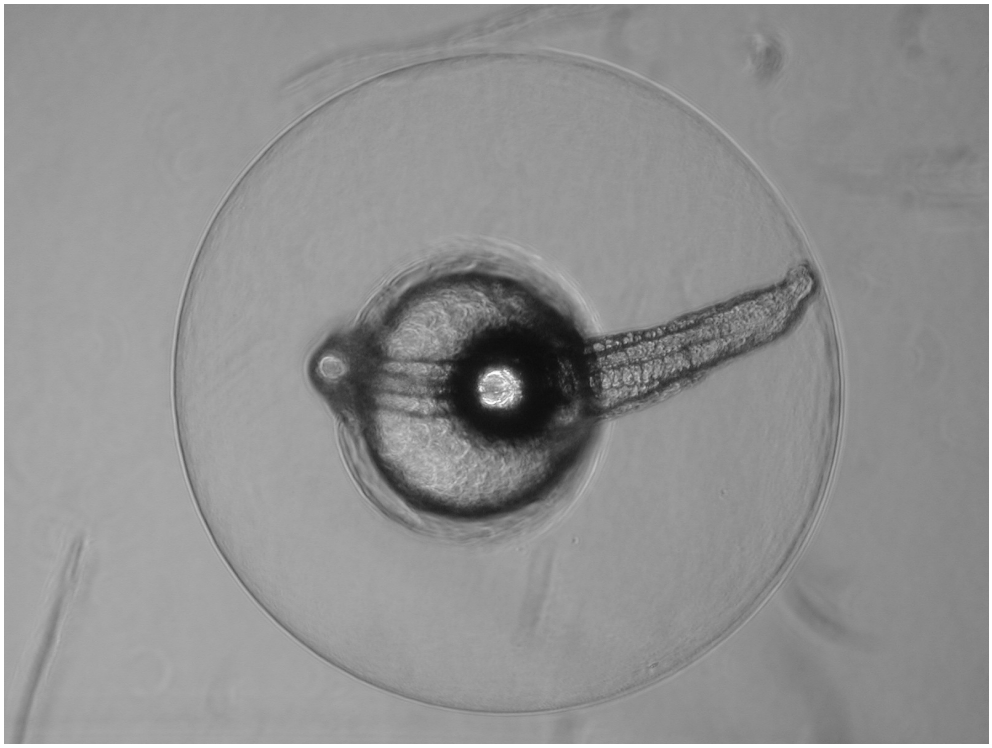
CHAPTER 5



Bred stretched eel embryo 35 hours post fertilisation

# Chapter 5

**Artificial maturation and reproduction of European silver eel:**

**development of oocytes during final maturation**

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*Keywords:* fish, physiology, endocrinology, hormonal stimulation, gonadotropin, 17,20β-dihydroxy-4-pregnen-3-one, ovulation, eggs, fertilisation, embryonic development, sperm motility

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CHAPTER 5

ABSTRACT
Attempts on artificial maturation of European eel (*Anguilla anguilla*) have largely been unsuccessful. The moment of stimulation of final maturation and ovulation is mainly based on weight increase related to the hydration response of the oocytes, which, in the European eel, is irregular. In contrast to Japanese eel, European eels show wide individual variability and much slower response to hormonal stimulation. In this study, the oocyte development of wild European silver eels was followed during final maturation. We describe 7 developmental stages based on 6 parameters: transparency, position and visibility of the nucleus, diameter of the oocyte, and diameter and number of oil droplets. Together, these parameters describe unidirectional changes from immature to over-ripe eggs. The developmental status of the gonads can thus be determined from biopsies. Of 23 female eels, 14 ovulated and were stripped, and 9 gave eggs that could be fertilised. Oocytes mature asynchronously, but this seems to be an artefact since fertility dropped with every new generation. As the timing of ovulation is crucial for fertility of the eggs, our developmental index of oocytes may result in more successful maturation protocols.

## INTRODUCTION

Artificial reproduction of Japanese eel (*Anguilla japonica*) became successful with the application of 17, 20 β-dihydroxy-4-pregnen-3-one (DHP) for final maturation and ovulation resulting in fertility and hatching rates of 89.6 and 47.6% respectively (Ohta et al., 1996). DHP was found the most effective steroid for the induction of final maturation in at least eight different fish species (Goetz, 1983; Nagahama, 1987). DHP was also found to induce predictable *in vitro* ovulation of yellow perch (*Perca flavescens*) oocytes (Goetz & Theofan, 1979). This is probably mediated by an effect on prostaglandin synthesis (Goetz, 1983). The latter has been reported to stimulate *in vitro* ovulation of pike (*Esox lucius*) and European eel oocytes (Jalabert, 1976; Epler & Bieniarz, 1978; Epler, 1981). In Japanese eel, DHP was found to induce both final maturation (Yamauchi & Yamamoto, 1982) and ovulation of oocytes (Yamauchi, 1990).

Lokman & Young (2000) used Ohta's (et al., 1996) protocol on New Zealand freshwater eels (*A. dieffenbachii* and *A. australis*). They obtained larvae of *A. australis* and kept them alive for a few days. Recently, Pedersen (2003, 2004) applied variations of the same protocol on European eel and obtained a few larvae that stayed alive for 2 days. Those larvae showed, however, delayed hatching and abnormal morphology. In addition to body weight increase during final maturation Ohta et al. (1996) and Pedersen (2003) used changes in diameter and appearance of oocytes as additional parameters for initiating ovulation. Four oocyte stages were described (Pedersen, 2003): stage 1 (small, black non-transparent cells); stage 2 (larger eggs with a dark-grey cytoplasm containing numerous, small dark oil droplets); stage 3 (the greyish cytoplasm and the oil droplets are more transparent, oil droplets with increased diameter and decreased numbers); stage 4 (migratory nucleus with cytoplasm as well as oil droplets highly transparent). The different stages were, however, not described in detail nor quantified. Although both authors mentioned asynchronous oocyte development, this was not quantified. Time of priming and induction of ovulation may, however, determine fertility.

Low fertility and hatching rates are not restricted to European eel, but are also found with other commercially important fish species, notably marine fish such as Atlantic halibut *Hippoglossus hippoglossus* (Nordberg et al., 1991; Holmefjord et al., 1993; Bromage et al., 1994), sole *Solea solea* (Houghton et al., 1985), turbot *Scophthalmus maximus* (Bromley et al., 1986), gilthead seabream *Sparus auratus* (Carrillo et al., 1989) and some salmonids (Bromage et al., 1992). In this study, we artificially induced maturation of male and female European silver eel from Lake Grevelingen (the Netherlands). Cytological changes during oocyte maturation were studied and categorised. An identification key of oocyte maturation is presented and used to describe final stages of female eel maturation.

## METHODS

*Experimental animals and period of treatments*

Silver eels (male and female) were caught in the fall of 2001 and 2002 during their seaward migration in the brackish Lake Grevelingen (Bout, Bruinisse, The Netherlands) at the North Sea sluice at 32 ppt. After arrival in the lab they were tagged with small passive transponders (TROVAN, EID Aalten BV, Aalten, The Netherlands).
Animals were treated from March 28 until August 5, 2002 (experiment 1) and from January 15 until July 2, 2003 (experiment 2). Experiment 1 was started with 51 males (100-150 g) and 32 females (83.1 ± 7.8 cm, 1160 ± 360 g). Experiment 2 was started with 100 males (100-150 g) and 30 females (72.7 ± 6.0 cm, 733 ± 180 g).

*Animal housing & welfare*

Males were kept in two 180-l tanks connected to a 2200-l recirculation system in artificial seawater (35 ppt, 18°C) under a 12/12-h light/dark regime. Females were kept in a 1500-l tank connected to a 2400-l recirculation system in artificial seawater (35 ppt, 18°C) under dark conditions. PVC pipes were added to serve as shelter. Both males and females were starved throughout the experiments. All fish received weekly treatments with antibiotics (Flumequin; Flumix, Eurovet, Bladel, The Netherlands, both of 50 $mgl^{-1}$ for 1-2 h). Wounds were sealed with solutions of silver nitrate (1%) and potassium dichromate (1%).

*Hormonal treatment protocol*

Males were anaesthetised weekly (benzocain, 80 ppm) and injected IP with 125 IU Human Chorionic Gonadotropin (HCG; Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands). Males were checked for spermiation by hand stripping. A drop of sperm was collected in a syringe (1-ml) and mixed with artificial seawater from the holding tanks. Sperm motility was estimated using a microscope. The day before fertilisation, three to five males displaying high sperm motility were selected per female and were IP injected with a single booster dose of 1000 IU HCG (Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands). Selected males were transferred to a 500-l tank with water of 20 °C.

Females were weekly anaesthetised (benzocain, 80 ppm) and injected IP with 20-mg carp pituitary extract (CPE; 'Catfish', Den Bosch, The Netherlands). From week 7

onwards, females were weighed two days after injection to determine the body weight index (BWI= body weight/initial body weight x 100). At the final stage, a female was primed by IP injecting a double dose of CPE (Lokman, personal comment). This is in contrast to the single dose primer that Ohta (et al., 1996) and Pedersen (2003) applied. Ovulation was induced by injecting a DHP-solution (2 mg DHP per kg female/175 µl 100% ethanol 1/1 diluted with buffered saline solution) at 8 locations in the ovary. DHP was injected at 21.00 h with the aim of ovulation occurring on the following day. After DHP injection, the female was transferred to a 1000 l tank in a 7000 l re-circulating system with water of 20 °C (2 °C increase; Ohta et al., 1996).

*Oocyte development during final maturation and ovulation*

Weekly biopsies of the ovary were taken when females started showing larger and softer abdomens. Additional biopsies were made at the time of priming, DHP injection and ovulation, respectively. Oocytes were sampled from a standardised location in the body (5 cm rostral to the genital pore) using an injection needle with an inner diameter of 1.2 mm. Freshly obtained oocytes were observed by phase contrast microscopy (NIKON Eclipse TS100) and photographed with a digital camera (NIKON Coolpix 4500). For measuring diameters of spherical oocytes and fat droplets, a 100x0.01=1 mm standard (Graticules LTD., Tonbridge, Kent, England) was photographed at same magnification. Diameters were measured after using UTHSCSA Image Tool 2.0 on photographs of fresh material. After microscopy and photography, oocytes were preserved in 4% buffered formalin.

*Hand-stripping and fertilisation*

Occurrence of ovulation was checked between 10 and 24 h after DHP injection. When eggs could be stripped easily, a sample was collected, observed, photographed and preserved as described. Then, males were stripped first. After collecting the sperm of 3 males (1-11 ml per male) motility was estimated by eye. Then the ripe female was anaesthetised and hand-stripped. The abdomen was kept dry and the released oocytes were collected in plastic Petri dishes with only a single layer of oocytes on the bottom. In the first experiment, females were stripped multiple times on the day of ovulation when possible. In the second experiment, females were stripped only once. Oocytes and sperm were mixed with a feather. Artificial seawater was added and the mixture was gently shaken for 30 s (Tanck, personal comment). Within 24 hours after ovulation, females were killed and the remaining gonad was weighed. The GSI was estimated calculating gonad weight/bodyweight x 100, corrected for stripping.

*Statistics*

Results were calculated and plotted as mean ± standard deviation. Significance in difference between oocyte diameter, fat droplet diameter and number of fat droplets was tested for each type of oocyte vs. the previous type and for each biopsy vs. the previous one with GLM (General Linear Model) repeated measures of SPSS 10.0 for Windows.

## RESULTS

### *I Artificial maturation and reproduction*

*Male maturation*

In both experiments, some males began spermiating after 6 weekly injections. After 7-9 weekly injections, more than half of the males were spermiating lasting for the period of treatment of 25 weekly injections. Selected males for stripping showed sperm motility percentages between 30-50%. At the time of stripping (15-29 h after HCG booster injection) these percentages had increased up to 80-90%. After activation with seawater, sperm motility ceased within 1 minute.

*Female maturation*

In the first experiment twelve females died during maturation and three females did not show weight increase within 19 weeks. The remaining seventeen females (53.1% of total number of animals) fully matured within 19 weeks (Table 1). Three of these females died showing a decreasing BWI after peaking. During the second experiment twenty-four females died without fully maturing. These deaths were, however, probably due to a virus infection as they had red abdomens and ventral fins. The remaining six females fully matured (Table 1).

**Table 1** Final maturation of 23 European female eels with exp.; experiment 1 or 2, inj.; the number of weekly CPE injections (exc. primer and DHP), tag; PIT-tag code, $W_i$; initial weight, $BWI_{1/2}$; BWI at moment 1 (priming) or 2 (DHP injection), $ECO_{1/2/3}$; the occurrence of an external cluster of extruded oocytes at the end of the oviduct at moment 1 (priming), 2 (DHP injection) or 3 (ovulation), t; the hours after DHP injection when ovulation started, GSI and fate of the females (for more explanation see text).

| exp | inj | tag | Wi | $BWI_1$ | $BWI_2$ | $ECO_1$ | $ECO_2$ | $ECO_3$ | t (h) | GSI | fate |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 13 | EB3C | 853 | 120.0 | 131.3 | no | no | yes | 14.5 | 37.6 | stripped and fertilised |
| 1 | 15 | 32A7 | 1262 | 112.8 | | yes | yes | yes | 14 | 41.3 | stripped and fertilised |
| 2 | 15 | 6C26 | 784 | 109.4 | 109.9 | yes | yes | yes | 14 | 43.5 | stripped and fertilised |
| 1 | 16 | A162 | 944 | 110.3 | | no | no | yes | 24 | 38.7 | stripped and fertilised |
| 1 | 17 | 6DEC | 1062 | 113.9 | 121.8 | no | yes | yes | 13 | 44.9 | stripped and fertilised |
| 1 | 18 | ECC9 | 785 | 118.0 | 129 | no | yes | yes | 13 | | stripped and fertilised |
| 1 | 19 | 514E | 1136 | 111.0 | 119.8 | no | no | yes | 13 | | stripped and fertilised |
| 2 | 23 | F9FE | 914 | 111.9 | 114.2 | no | yes | yes | 14.5 | 45.6 | stripped and fertilised |
| 2 | 25 | 8FDF | 604 | 117.0 | 120.5 | yes | yes | yes | 13.5 | 48.9 | stripped and fertilised |
| 1 | 12 | 6673 | 1175 | 118.1 | 118.1 | yes | yes | yes | <18 | 51 | stripped, no fertilisation attempted |
| 1 | 14 | EA57 | 1204 | 111.6 | 114.1 | no | no | yes | 24 | 46.9 | stripped, no fertilisation attempted |
| 1 | 14 | OC7O | 968 | 119.1 | 121.2 | no | no | yes | <18 | 43.3 | stripped, no fertilisation attempted |
| 1 | 16 | FD1A | 1002 | 124.9 | | no | yes | yes | 10 | 36.3 | stripped, no fertilisation |
| 1 | 16 | FCB8 | 1714 | 137.8 | | yes | yes | yes | 10 | 60 | stripped, no fertilisation |
| 1 | 15 | F184 | 1290 | 130.4 | | yes | yes | yes | | 41.6 | no ovulation |
| 1 | 15 | FAOB | 726 | 104.1 | | yes | yes | yes | | 28.2 | no ovulation |
| 1 | 17 | O189 | 1005 | 106.9 | 117.5 | no | no | no | | | no ovulation |
| 1 | 18 | E431 | 795 | 114.7 | 121.8 | no | yes | yes | | | no ovulation |
| 1 | 14 | OD51 | 1112 | 103.7 | | no | | | | 35.8 | died during final maturation |
| 1 | 15 | O4FD | 889 | 102.4 | | yes | | | | | died during final maturation |
| 1 | 16 | E9C1 | 1202 | 96.3 | | yes | | | | | died during final maturation |
| 2 | 18 | 1692 | 715 | 113.9 | 119.8 | no | no | | | 39.2 | died during final maturation |
| 2 | 20 | 3E61 | 966 | 111.3 | | | | | | | died during final maturation |

*The final stage of female maturation and ovulation*

During experiment 1, fourteen females were primed, and four did not ovulate (Table 1). Females F184 and FAOB showed dilation at priming. Dilation caused formation of an external cluster of extruded oocytes (ECO) and other gonadal material at the end of the oviduct. Female E431 had oocytes with single fat droplets or burst open at DHP injection and was considered over-ripe (Sugimoto et al., 1976). Ten females successfully ovulated (71.4% of surviving animals) and were stripped. During experiment 2, six females were primed. Female 3E61 died after priming (Table 1). Female 1692 died after DHP injection (Table 1). Four females successfully ovulated and were stripped. Thus, in total, fourteen females were stripped (Table 1). These eels matured between 12-25 injections (16.6 ± 3.7). They showed a BWI of 117 ± 8 (range 109-138) at priming and 120 ± 7 (range 110-131) at injection of DHP (Table 1). They had a GSI of 44.8 ± 6.5 (range 36.3-60.0). Females ovulated between 10 and 24 h after DHP injection in a quite narrow range of 13 to 14.5 h after DHP injection (14.8 ± 4.6). The weight increase between priming and DHP injection varied between 0 and 11.3% (Table 1). Of the 14 stripped females 9 were fertilised. BWIs of these females are depicted in Figure 1. From the other five females, FD1A and FCB8 were over-ripe (Sugimoto et al., 1976) and for three, fertilisation was not attempted because spermiating males were still lacking at that time.

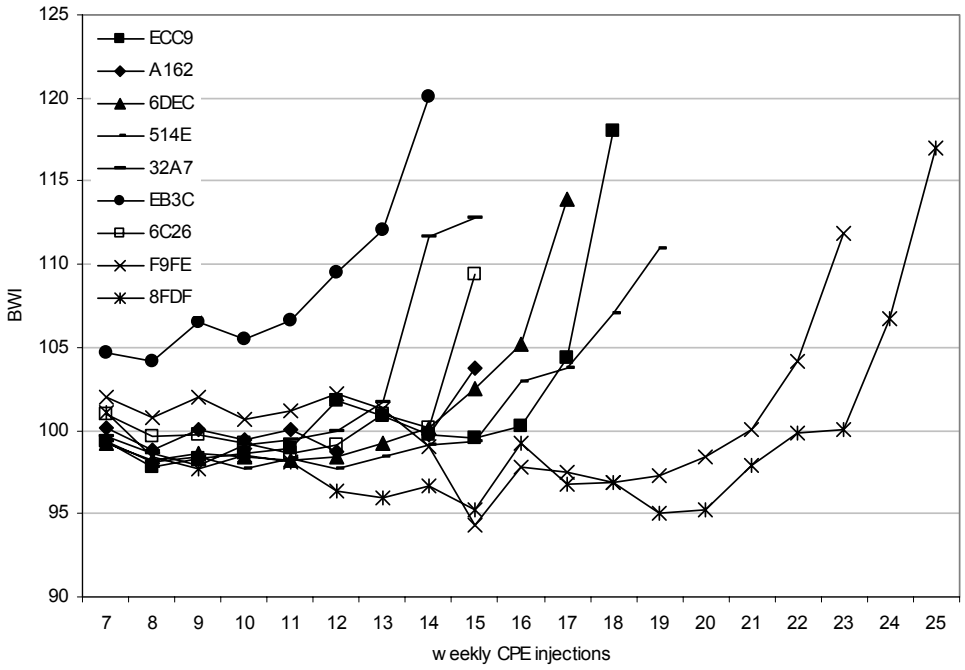


**Figure 1** Body weight index (BWI) of maturing female eels with fertilised oocyte batches versus the number of weekly injections.

*Fertilisation, cleavage and embryo formation*

No attempt was made to fertilise those egg batches obtained by stripping first ovulating females in experiment 1. This is because the males were not spermiating yet at that time. Samples from females FD1A and FCB8 showed that all oocytes were over-ripe (Sugimoto et al., 1976), and fertilisation was not therefore attempted. Fertilisation was attempted and established for oocyte batches from the remaining nine females (Table 1). After transfer to rearing tanks, more than 90% of the eggs from all different batches floated. Sinking eggs soon turned white and were removed. During the first three hours after fertilisation (at 20˚C), eggs from 9 females showed early stages of development (Figure 2a). Most eggs showed meroblastic cleavage up to the eight-cell stage. Later cell divisions became difficult to observe since the percentage of surviving eggs was rather low. Egg batches of females F9FE and 6DEC, however, resulted in the development of about 1500 and 100 embryos respectively (Figure 2b). Embryonic development continued until 100 hours after fertilisation when last embryos died (Palstra et al., 2004b). Hatching was not observed.

a)

b)

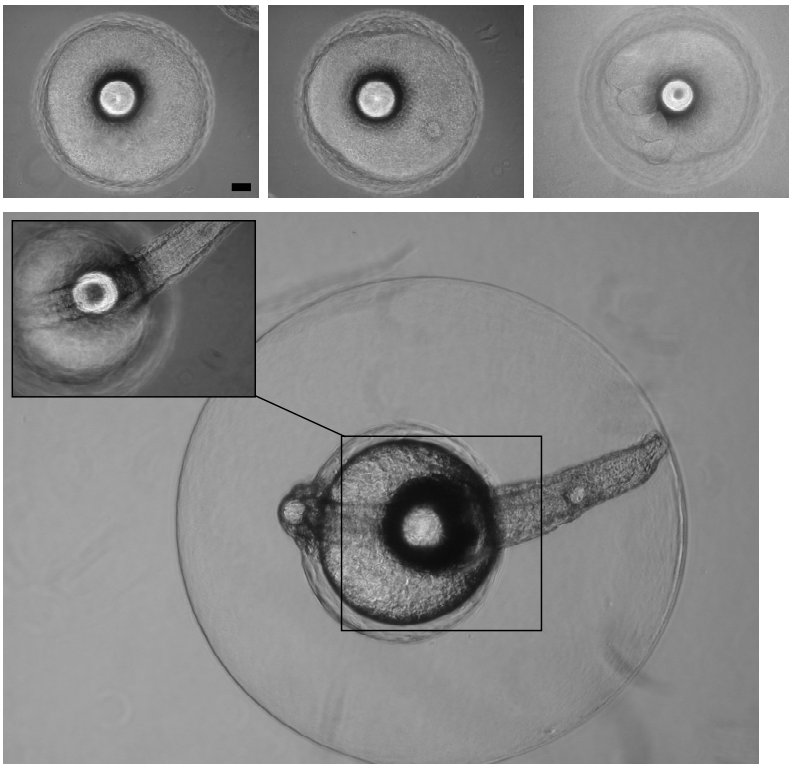

**Figure 2** a) Activated eggs within 3 hours after fertilisation in 9 fertilised egg batches (scale bar = 100μm) with first stages of meroblastic cleavage. b) Stretched embryo with developed somites (see insert) at 32 hours after fertilisation reared at 20˚C. (Phase contrast microscopy)

CHAPTER 5

## *II Development of oocytes during final maturation*

### *Appearance of oocytes during final maturation*

In the different biopsies 9 different types of oocytes could be distinguished:

a) Non-transparent, small oocytes.
b) Partially transparent oocytes with a visible central nucleus surrounded by numerous small fat droplets.
c) Larger, fully transparent oocytes with nucleus mostly not visible and with larger fat droplets in the centre.
d) Fully transparent oocytes with the nucleus between centre and periphery (GVM).
e) Fully transparent oocytes with the nucleus at the periphery and larger fat droplets starting to cluster opposite it.
f) Fully transparent oocytes with the nucleus still at the periphery and with even larger fat droplets now completely clustered opposite it.
g) Fully transparent oocytes with no visible nucleus and few large fat droplets.
h) Fully transparent oocytes with no visible nucleus and a single fat droplet.
i) Turbid oocytes with a single fat droplet.

Table 2 lists measurements of oocyte diameters, fat droplet diameters and number of fat droplets found in these types. Every type was significantly different from the previous one for at least one parameter except for type f vs. e, which however differed in number of fat droplets with 100%.

### *Final stages of oocyte development*

Some oocyte characteristics from sequential biopsies of female F9FE (which egg batches showed embryonic development) during final maturation are illustrated in Figure 3 and 4. Figure 3a shows that BWI increased over time with 20%. The percentage transparency increased from 24 at s1 to 53% at s2 but decreased thereafter (Fig. 3b). Oocyte diameters increased only in the first samples s1 and s2 (Fig. 3c) and showed that transparency coincided with hydration. Fat droplet diameters increased while at the same time the number of fat droplets decreased from about 190 in the first biopsy to a few in the last (Fig. 3d). Fat fusion was observed and followed directly in time (Fig. 4). A fusion rate of 7.1 fat droplets per hour was found. Figure 5 shows that the first sample taken 2 weeks before priming (s1) contained mainly type b-c oocytes. Sequential samples taken 1 week before priming (s2), at priming (s3) and at DHP injection (s4) contained all types but less type b and more type g-h. The final sample at stripping (s5) mainly contained type g-h.

Thus, five processes hydration, transparency, fat fusion, GVM and GVBD were found to proceed in time. Hydration occurs in the first stage of final maturation, while fat fusion could be observed to develop over several weeks. As GVM and particularly GVBD are characteristic for the last phase, the 5 processes should describe the development of the oocyte. Using these keys we categorised the distinguished types of oocytes into seven developmental stages of final oocyte maturation (Fig. 6).

**Table 2** Parameters of different types of mature oocytes (a-h). Panel A: oocyte diameters. Panel B: fat droplet diameters. Panel C: number of fat droplets per oocyte. Mean, standard deviation (stdev) and range are given and the number of measurements on fat droplets or oocytes from particular samples of particular eels. Significance levels are given for each type vs. the previous type. *** Denotes a significance of P<0.001, ** of P<0.01, * of P<0.05, 0 of P>0.05 and – indicates too few data to test. (For more explanation see text)

**a)**

| type | oocyte diameter (µm) mean | stdev | range | n oocytes | n samples | eels | sign. |
|---|---|---|---|---|---|---|---|
| a | 451 | 117 | 316-644 | 35 | 3 | F9FE, 8FDF | |
| b | 653 | 70 | 532-776 | 29 | 2 | F9FE, 8FDF | *** |
| c | 790 | 38 | 723-864 | 29 | 4 | F9FE, 8FDF | *** |
| d | 797 | 29 | 757-847 | 11 | 3 | F9FE, 8FDF | 0 |
| e | 826 | 39 | 784-897 | 11 | 4 | F9FE, 8FDF | ** |
| f | 827 | 45 | 767-890 | 6 | 4 | F9FE, 8FDF | - |
| g | 831 | 38 | 716-887 | 25 | 4 | F9FE, 8FDF | 0 |
| h | 800 | 61 | 675-922 | 32 | 4 | F9FE, 8FDF | 0 |

**b)**

| type | fat droplet diameter (µm) mean | stdev | range | n fat droplets | n oocytes | n samples | eels | |
|---|---|---|---|---|---|---|---|---|
| a | | | | | | | | |
| b | 32.5 | 15.9 | 10.0-61.1 | 40 | 2 | 1 | 8FDF | |
| c | 42.9 | 14.5 | 15.0-81.0 | 64 | 4 | 2 | F9FE | ** |
| d | 39.9 | 18.7 | 11.6-140.3 | 92 | 5 | 3 | F9FE, 8FDF | * |
| e | 60.3 | 25.5 | 27.6-162.1 | 83 | 8 | 2 | F9FE | *** |
| f | 80.9 | 44.1 | 16.4-202.1 | 115 | 7 | 3 | F9FE, 8FDF | 0 |
| g | 97.5 | 61.7 | 18.4-311.7 | 78 | 6 | 2 | F9FE, 8FDF | * |
| h | 343 | 12.4 | 326.2-354.6 | 4 | 4 | 2 | F9FE, 8FDF | *** |

**c)**

| type | number of fat droplets mean | stdev | range | n oocytes | n samples | eels | |
|---|---|---|---|---|---|---|---|
| a | >200 | | | | | | |
| b | >200 | | | | | | |
| c | 215 | 22 | 183-233 | 4 | 2 | F9FE, 6C26 | |
| d | 94 | 22 | 90-98 | 4 | 2 | F9FE, 6C26 | - |
| e | 115 | 33 | 100-130 | 6 | 2 | F9FE, 6C26 | - |
| f | 52 | 22 | 34-39 | 6 | 2 | F9FE, 6C26 | - |
| g | 19 | 2 | 11-25 | 10 | 4 | F9FE, 6C26 | *** |
| h | 1 | 0 | | 10 | 4 | F9FE, 6C26, EB3C | *** |

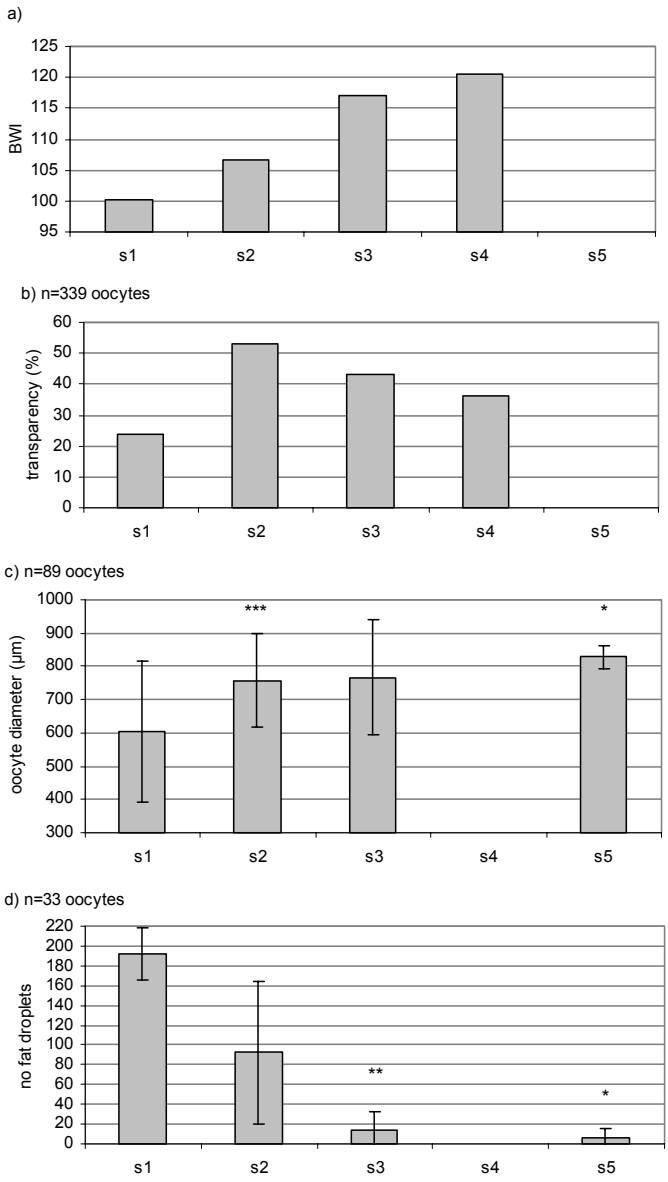


**Figure 3** Developmental characteristics of oocytes at final maturation from a single female (F9FE). a) BWI, b) percentage of transparent oocytes, c) oocyte diameter, and d) number of fat droplets. Sample moments are given on the x-axis: s1= 2 weeks before priming, s2= 1 weeks before priming, s3= at priming, s4= at DHP injection and s5= at stripping. Changes in oocyte diameter and number of fat droplets were statistically tested. Significance levels are given for each biopsy vs. the previous one. *** Denotes a significance of $P<0.001$, ** of $P<0.01$, * of $P<0.05$.

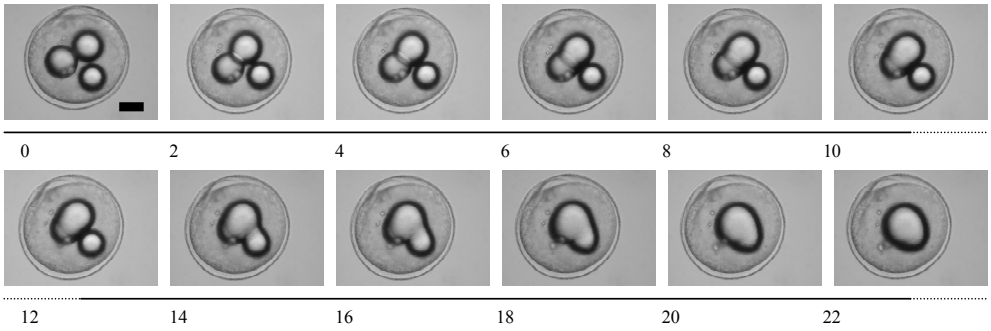


**Figure 4** Fat droplet fusion followed in time within a single water activated oocyte (scale bar = 250μm). The axis gives the 2-minute time lapse between each picture.

**Developmental stages of eel oocytes during final maturation**

**Stage 0** opaque oocytes (Table 2: type a).
**Stage 1** opaque oocytes with a centred nucleus becoming visible (Table 2: type b).
**Stage 2** fully transparent oocyte; fat droplets clustered (Table 2: type c).
**Stage 3** fully transparent oocyte with GVM (Table 2: type d).
**Stage 4** fully transparent oocyte with nucleus at periphery (Table 2: type e).
**Stage 5** fully transparent oocyte with nucleus at periphery with few large fat droplets (Table 2: type f).
**Stage 6** fully transparent oocyte with GVBD; few fat droplets (Table 2: type g).
**Stage 7** fully transparent oocyte with GVBD; single fat droplet (Table 2: type h).

*Appearance of stripped oocytes*

Most DHP injected females could be stripped easily resulting in large quantities of transparent oocytes (1007 ± 55 μm, n=7 oocytes from female F9FE). These contained few large fat droplets (137 ± 84 μm, n=538 fat droplets from female F9FE) and the nucleus was not visible (GVBD). Some females (32A7, 6C26, A162) however, still contained large numbers of oocytes (resp. 37.5, 93 and 62%) in which the nucleus was still visible. Of two females (EA57, OC7O) only a small number of oocytes could be stripped containing many small fat droplets and GVM.

## DISCUSSION

*Male maturation*

In this study, spermiation of European eels started after 5 weekly injections. Already one week later high motility sperm was obtained. Pedersen (2003) found spermiation to start after 4 weekly HCG injections and Müller et al. (2002) also after 5 weekly injections. Ohta & Unuma (2003) found the first spermiation in Japanese eel after 5-6 weekly injections. High motility sperm was obtained early in comparison with results of Perez (et al., 2000) who obtained this only after 10 injections. In our study males showed

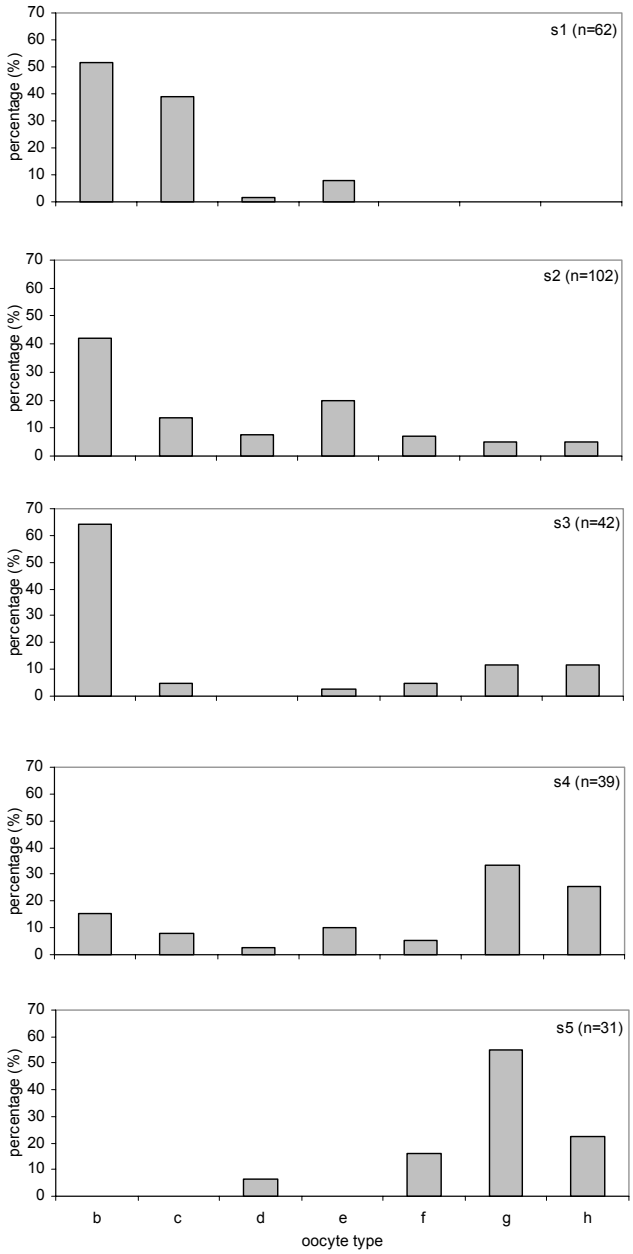


**Figure 5** Percentage of oocyte types at final maturation in sequential biopsies (s1-5) from a single female (F9FE) with s1= 2 weeks before priming, s2= 1 weeks before priming, s3= at priming, s4= at DHP injection and s5= at stripping. A gradual shift is displayed from type b-c at s1 to type g at s5.

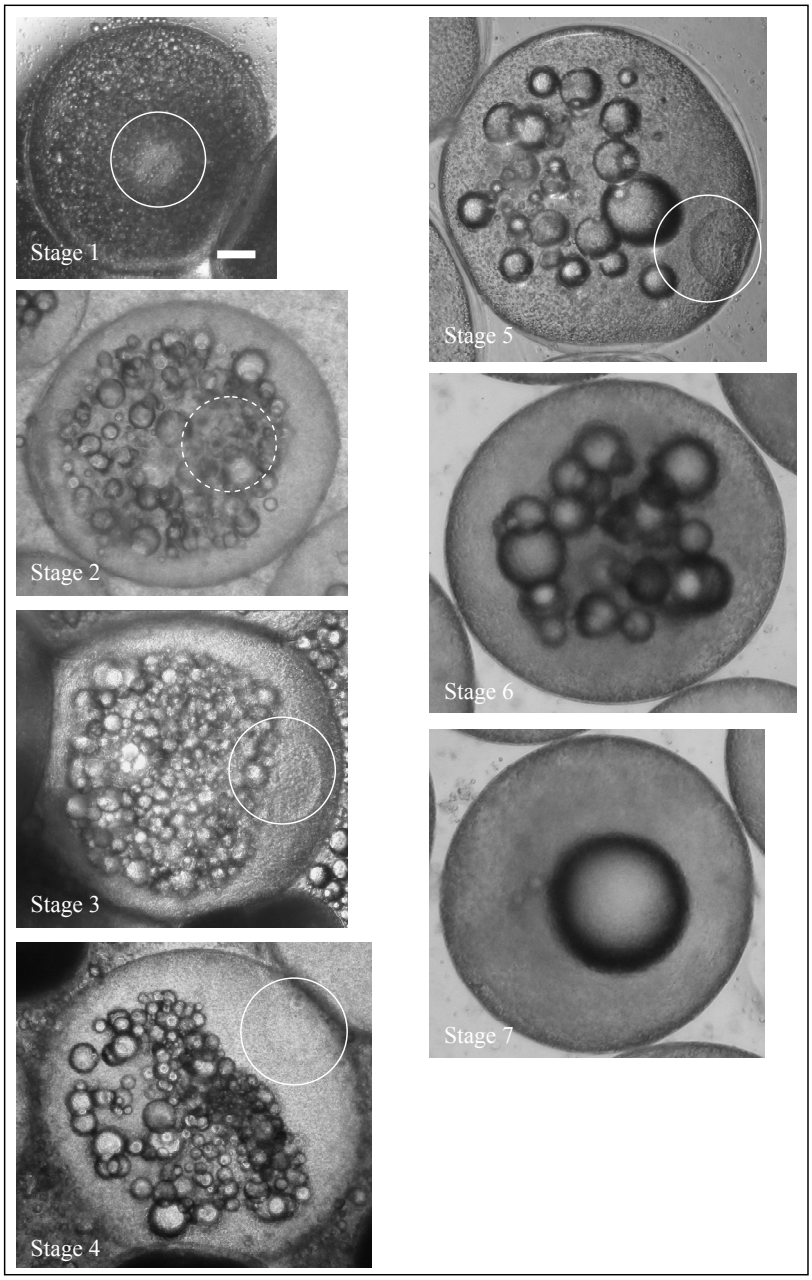


**Figure 6** Seven developmental stages in oocyte maturation (scale bar = 100µm). Encircled are the positions of the migrating GV. (Stage 1-5 phase contrast microscopy, stage 6-7 light microscopy). For more explanation see text)

moderate motility (30-50%) and high motility sperm (80-90%) only after a booster dose of 1000 IU HCG. Using farmed eels, Pedersen (2003) obtained sperm motility close to 100% without a booster injection. During the whole experimental period of 6-25 weekly injections, males could be selected with high motility sperm. Sperm was successfully applied for artificial fertilisation within 5 minutes of stripping. The motility of eel sperm after activation was observed to continue for 30-60 s under the microscope, which is comparable to that recorded for most other teleosts (Coward et al., 2002).

*Female maturation*

Mortality among experimental females was high in the first and second experiment at 37.5% and 80%, respectively. Similar or higher mortalities were found by other research groups but were not reported (personal comments Durif, Pedersen, van Ginneken). Fourteen females were stripped between 12-25 weekly injections. This timing is comparable to that reported by Pedersen (2003) who found maturation after 24-25 weekly injections with wild European eels (n=3: 623-837 g) and 14-22 weekly injections with farmed European eels (n=9: 571-820 g) using a comparable dose of salmon pituitary extract (SPE). Ohta (et al., 1996) reported a range of 9-12 weekly injections with farmed Japanese eels in a weight range of 701-980 g with SPE. The maturation response of European and Japanese eels is depicted in Figure 7. European eel thus shows both a delayed as well as a more extended response in comparison to Japanese eel. These differences seem to be species specific and not a matter of wild vs. farmed eels, weight or the source of the pituitary extract (CPE or SPE). In addition to a highly variable response time we also observed that the body weight increase of European eels is highly variable. From Figure 1 is evident that the slopes of BWI vs. time can be both low and steep. Japanese eel respond also in this matter in a more uniform way. The BWI of Japanese eels increases from 100 to above 110 in one week (Ohta et al., 1996). Thus the increase in female bodyweight is used as a reliable indicator of the last phase of ovarian maturation of Japanese eel (Yamamato et al., 1974; Sugimoto et al., 1976; Oka 1979; Wang et al., 1980; Yamauchi and Yamamoto, 1982; Satoh et al., 1992; Tachiki and Nakagwa, 1993). It appears that a similar procedure is not applicable for European eel (this study, Pedersen 2003). The other approach to predict the right time for final maturation can be the evaluation of the developmental stages of the oocytes in the ovary.

*Oocyte maturation*

Non-transparent oocytes that are found until final maturation are small and fully filled with fat droplets, which are products of the secondary yolk / midvitellogenic stage (Adachi et al., 2003). Fast growth and increase in transparency occurs in the tertiary yolk stage / late vitellogenic stage (Adachi et al., 2003). The latter is considered as a result of fusion of yolk globules (reviewed in Wallace and Selman, 1981). Oocytes now undergo their final maturation during which the chromosomes resume meiosis and proceed to the second meiotic metaphase with the concomitant formation of the first polar body (Goetz, 1983). The increase in transparency coincides with swelling of the oocytes due to hydration (up to 800-900 µm in this study). Pronounced hydration up to 30% between opaque oocytes of maximum diameter and fully transparent oocytes was observed in this study which corresponds with other marine teleosts spawning pelagic eggs (Wallace and Selman, 1981).

European eel spawns up to 4 million eggs which are not sticky and which rise to the water surface with a speed of over 2 meters per hour (van Ginneken et al., 2005a). Simultaneous with hydration we observed also fusion of fat droplets (Figs. 3 and 4), which in all cases caused a reduction from >200 to a few droplets (10-1).

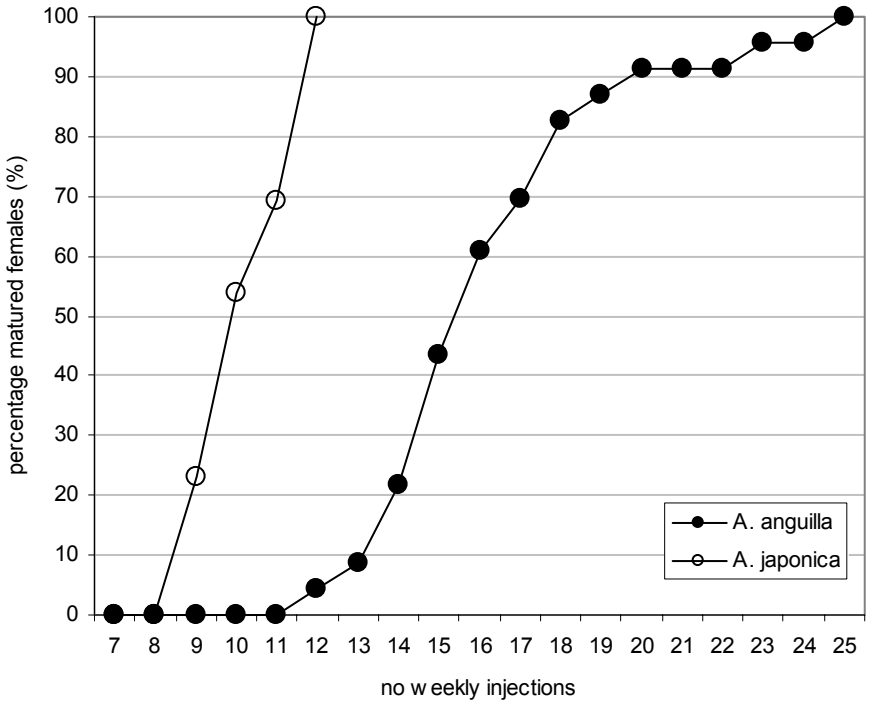


**Figure 7** Frequency of occurrence of matured females of *A. anguilla* (this study) vs. *A. japonica* (Ohta et al., 1996). *A. anguilla* shows a much slower response to injection of pituitary extract.

Some discrepancy exists between different authors with respect to the diameter of the European eel egg (Boetius & Boetius, 1980). In literature a wide range of diameters is reported. In most cases the stage of the oocyte was not clear and therefore comparison is restricted to results of Pedersen (2003) and Japanese eel. Ohta (et al., 1997) and Adachi (et al., 2003) mention diameters of oocytes at GVM of 700-800 µm in Japanese eel. Oocytes at GVM in European eel have diameters of 800 µm (this study). Pedersen (2003) reports diameters of 700-850 µm. Adachi (et al., 2003) mentions diameters of oocytes where the germinal vescicle reaches the periphery of 850-900 µm. Oocytes from European eel in this stage are 750-950 µm (this study). Pedersen reports diameters of 750-860 µm. No significant differences seem to exist between the two species. In this study, oocyte hydration occurred only up to stage 2. Oocyte diameter did not continue to change significantly indicating that further hydration stopped. Therefore, we can conclude that the oocyte diameter does not suffice as discrimination tool for oocyte development of European

eel. Until the time of spawning, the GV moves forwards to the periphery as the lipid droplets coalesce. Finally, prior to ovulation, the GV migrates a short distance to the surface of the oocyte after which it breaks down (GVBD). The time and rate at which the GV migrates to a peripheral position varies between species. In this study, oocytes showed GVM for about 48 hours although individual differences were high.

Fat droplets were measured and counted per individual oocyte stage (Table 2). Counting fat droplets in oocyte stage 1 was not possible because of limited transparency and high numbers (>200). Fat droplet numbers did not differ significantly between stages 3, 4 and 5 (GVM to periphery). Concerning lipid coalescence in higher teleosts, Goetz (1983) states that the degree of lipid coalescence follows a phylogenetic pattern. Lipid coalescence in ovulated oocytes of higher teleosts results in the formation of one major fat droplet (reviewed by Goetz, 1983). In contrast, ovulated oocytes in lower teleosts still contain a large number of lipid droplets (reviewed by Goetz, 1983). Goetz (1983) states that European eel, like Japanese eel, is a major exception to this trend in which one to several large lipid droplets are present in oocytes following GVBD (Epler and Bieniearz, 1978; Yamauchi and Yamamoto, 1974). Although this is true for most observed oocytes in this study, we also found oocytes with single fat droplets still containing a peripheral nucleus. Oocytes with single fat droplets soon turned over-ripe. Females peaking in BWI (females FD1A, FCB8) possessed large quantities of over-ripe oocytes and were not fertile. DHP sensitivity dropped since most over-ripe females could not be induced to ovulate (females F184, FAOB, E431). Soon after a peak in BWI females developed an ECO and ovulated spontaneously.

*Application of the oocyte maturation key*

The seven developmental oocyte stages were categorised in an identification key. This key was used to determine the average maturation stage of oocyte samples. Figure 8 shows average stages of individual females of which batches were fertilised. Individual variation in developmental speed is clear. Administration of a CPE booster causes a change of –0.3 up to 3.4 stages a day later. In most cases less developed batches showed greatest response. After DHP administration development in most cases continued either induced still by CPE or by DHP. Individual maturation in these females converges towards the moment of ovulation. On average oocyte maturation stage was 4.0 ± 1.2 at CPE injection, 5.1 ± 1.2 at DHP injection and females ovulated at 5.9 ± 0.5 ($P<0.01$ vs. stage at CPE injection). On average oocyte batches developed with speeds of 1.1 stage after CPE injection and 0.7 stage after DHP injection. Females 6DEC and F9FE of which eggs showed embryological development ovulated at average oocyte stage 5.9 reflecting fully transparent oocytes with GVBD and only few fat droplets.

*Synchronous or asynchronous ovarian development?*

In literature, eels are considered having synchronous ovaries typical for teleosts spawning once and then die (Wallace & Selman, 1981). In this study, ovaries showed asynchronous development of oocytes maturing over several generations (Figure 5). Most eels ovulated more than once over periods up to several days. These findings are supported by other authors concerning European eel (Bezdenezhnykh & Prokhorcik, 1984; Pedersen, 2003), New Zealand eel (Lokman & Young, 2000) and also Japanese eel (Pedersen, 2003).

However, as discussed before, we were not able to fertilise other than first stripped batches. Also, in some cases females were stripped almost completely empty. These females showed a large first generation oocytes. In the case of small early oocyte generations we attempted to induce ovulation of later ones although fertility dropped. These observations support the idea that asynchronous oocyte development has an artificial rather than a natural origin.

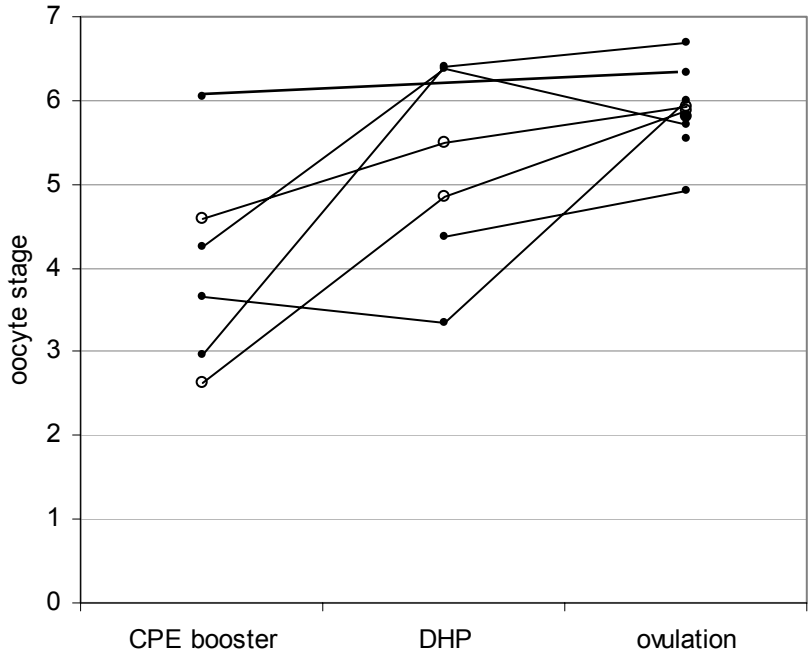


**Figure 8** Average oocyte developmental stages in biopsies of individual eels of which eggs were fertilised (lines) at CPE booster injection, DHP injection and ovulation. Lines connect samples from the same female. Open circles reflect batches that showed embryological formation.

*Oocyte stage and ovulation time*

In this study ovulation was induced between 10 and 24 h after DHP injection with most females (8 out of 14) in a quite narrow range of 13-14.5 h after DHP injection. Pedersen found comparable ovulation times between 13.5 and 17.5 h after DHP injection. Ohta (et al., 1996) and Kagawa (et al., 1997) found ovulating females of Japanese eel between 15 and 21 h after DHP injection, independent on circadian rhythm (Kagawa, 2003). It might be that oocytes of European eel are more sensitive to DHP than those of Japanese eel. Differences in oocyte appearance clearly exist between both species (Pedersen, personal comment). Differences in timing of ovulation are, however, at least partly, determined by the developmental stage and diameter of the oocytes. In this study we found indications of a negative correlation between timing of ovulation and development of

oocytes. Goetz & Theofan (1979) and Goetz (1983) confirm this, although the level of synchrony between DHP as inducer of final maturation and ovulation at the used dose is uncertain. Correlation between timing of ovulation and oocyte diameter was found by Ohta (et al., 1997). *In vitro* experiments on Japanese eel showed that oocytes between 700-800 μm were sensitive to DHP (Ohta et al., 1997). Oocytes over 800 μm in diameter became more sensitive to the steroid (Ohta et al., 1997). For DHP induced ovulation of Japanese eel, a minimum oocyte diameter of 750 μm is used as a criterion (Pedersen, personal comment). Oocytes of European eel in this and Pedersen's (2003) study were, on average, larger in comparison with Japanese eel (Kagawa et al., 1995; Ohta et al., 1997) at the time of DHP injection. Oocytes at the desired developmental stage need to be induced to ovulate within 17 hours after DHP injection since Ohta (et al., 1996) found fertility and also hatching rates decreasing rapidly after.
European eel shows a highly individual response in timing and speed of maturation in contrast to Japanese eel. Therefore, BWI is an unreliable indicator of the last phase of ovarian maturation of European eel. Hence other tools are necessary to quantify the maturation stage of oocyte samples. In this study, seven oocyte maturation stages were categorised in an identification key. We used this key to determine the average maturation stage of oocyte samples. The average stage, level of transparency and oocyte diameters proved to be useful complementary characteristics in quantifying the individual maturation status.

ACKNOWLEDGEMENTS
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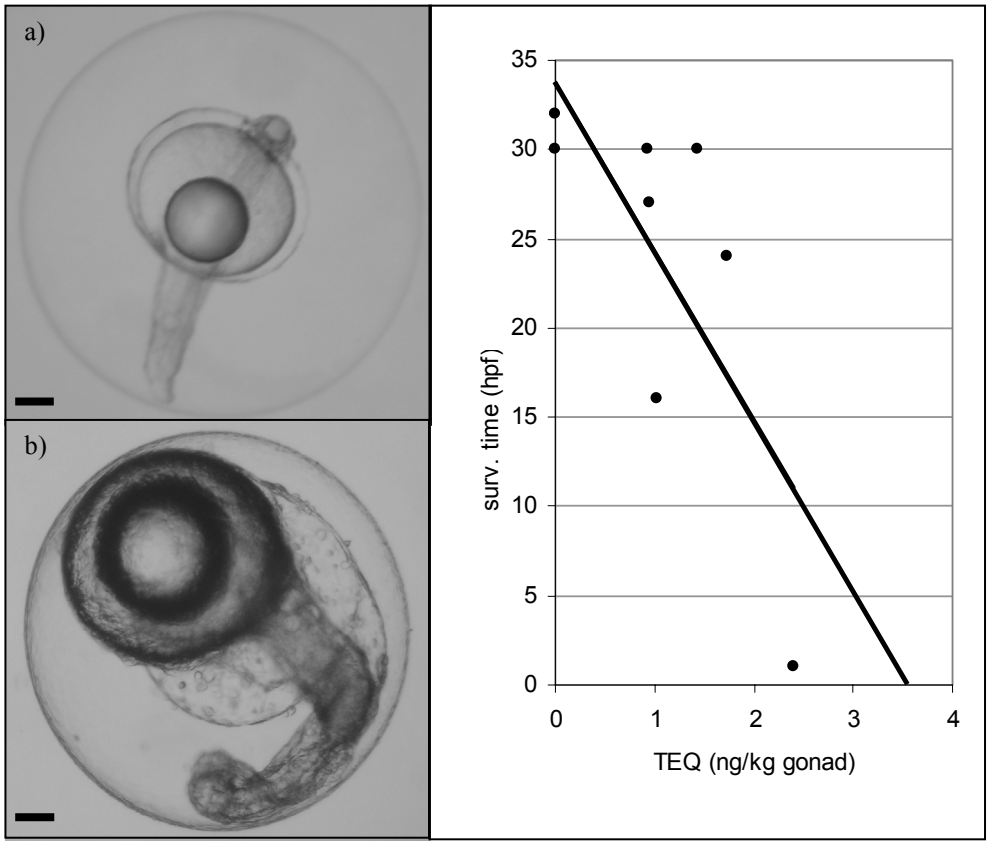


**Figure 2** a) Healthy European eel embryo at 30-35 hpf with heartbeat and yolk sac with large fat droplet, b) Larger embryo of an unhealthy batch at identical time of development displaying yolksac oedema, deformed head region and absence of heartbeat. Scale bars represent 100 µm. c) Negative correlation between total TEQ values (ng/kg gonad) and embryo survival time (hours post fertilisation) of fertilised eggs of 8 hormone induced, stripped females.

# Chapter 6

**Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama?**

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Keywords: Sargasso, fish, decline, migration, maturation, reproduction, spawning, contamination, teratogenicity, polychlorinated biphenyls PCBs, fertility, embryology

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CHAPTER 6

ABSTRACT
Eel populations world-wide are dangerously close to collapse. Our study is the first to show that current levels of dioxin-like contaminants are strong candidates, because of their devastating effects on development and survival of eel embryos. Female and male silver eels were artificially stimulated to maturation and reproduction by treatment with carp pituitary extracts and hCG respectively. During maturation of female European silver eels about 60 g fat per kg eel is incorporated in the oocytes. Together with the fat however, persistent organic pollutants such as dioxin-like polychlorinated biphenyls (PCBs) are incorporated too. The total dioxin-like toxic potency of the individual gonad batches was determined as TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxine) – equivalents (TEQs), using an in vitro reporter gene assay. The observed differences in development and survival showed a significant negative correlation with the TEQ levels in the gonads, already at levels far below the maximal allowable level for fish consumption i.e. 4 ng TEQ/kg fish. The clear inverse relationship between the TEQ-level and the survival period of the fertilised eggs strongly suggest that the current levels of dioxin-like compounds seriously impair the reproduction of the European eel. The peak of the environmental levels of dioxin-like PCBs and the decline of eel coincide world-wide, further suggesting that, in addition to other threats, these contaminants contributed significantly to the current collapse of eel populations.

## INTRODUCTION

Eel populations world-wide are dangerously close to collapse. The numbers of glass eels caught have declined by 99% since the early 80s (Anonymous 2003). Several anthropogenic factors implicated in the decline of European eel (*Anguilla anguilla*) are assumed to act before or during the eels' oceanic phase. Migrating (silver) eels do not feed 'en route' and are totally dependent on their fat stores to fuel migration and gonad development. With fat consumption however, internal concentrations of lipophilic pollutants rise, thus increasing the risk for toxic effects. Eels often reside in contaminated sediments and accumulate high levels of especially polychlorinated biphenyls (PCBs, van Leeuwen et al. 2002). These compounds have been shown to have adverse effects on fertility in fish (Stouthart et al. 1998) and amphibians (Gutleb et al. 1999) but also to disrupt mammalian oocyte maturation and follicle physiology in every species studied (Pocar et al. 2003). These effects are at least partially mediated via interaction with the aryl hydrocarbon receptor (AhR), which after binding is translocated to the cell's nucleus. There it interacts with dioxin response elements and disturbs physiological and developmental processes (Safe 1994).

In a recent review, Robinet & Feunteun (2002) stated that ecotoxicological studies on the reproduction capacity of contaminated eels were not available. Until now, it was not possible to study the effects of contaminants on fertility and embryonic development since artificial reproduction has been unsuccessful. Recently however, we have been able to fertilise eggs and follow embryonic development (Palstra et al. 2005). In these and subsequent trials, large differences were observed with respect to development in fertilised egg batches. We hypothesised that this was caused by maternal dioxin-like contaminants

deposited in the egg yolk. Therefore we measured in this study the levels of dioxin-like compounds in muscle and gonad tissues from these eels and correlated their distribution to embryonic development.

## MATERIAL & METHODS

Twenty-five female (500-1700g) and fifty male silver eels (100-150g) were caught in the wild during their seaward migration. All males and most females (n=18) were caught in Lake Grevelingen (Bout, Bruinisse, The Netherlands) and 7 females were caught in River Loire (France) in the fall (October-November) of 2001, 2002 and 2003. After transport to our laboratory, twelve females were immediately sacrificed as control animals. The remaining females were injected weekly with Carp Pituitary Extract (CPE: 20-mg/kg) and were not fed during the experimental period. Ovulation could be induced by injecting female eels with 17,20β-dihydroxy-4-pregnen-3-one (2-mg/kg) after which they were hand-stripped. Males were injected weekly with Human Chorionic Gonadotropin (125 IU/male) for at least 7 weeks, when sperm was collected to fertilise the eggs. Fertilised eggs were reared in artificial seawater at 20 ˚C as long as development proceeded. The protocol is described in detail by Palstra et al. (2005). Stripped females were sacrificed to determine age, based on otolith rings (Svedäng et al. 1998), and Gonadosomatic Index (GSI; relative gonadal weight). Lipid extraction of muscle and gonad tissue was performed as previously described by Murk et al. (1998).

Dioxin-like compounds, expressed as TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxine) – equivalents (TEQs), were determined by using a reporter gene assay DR-CALUX (Aarts et al. 1995). This assay is based on rat-hepatoma cells (H4IIE) stably transfected with a plasmid carrying the luciferase gene of fireflies (*Photinus pyralis*) as a reporter gene. The obtained TEQ-value is specific for dioxin-like compounds including PCBs as well as polyhalogenated dibenzo-dioxins/-furans (PCDD/Fs). However, dioxin-like PCBs are predominant in aquatic ecosystems (Murk et al. 1998; de Boer et al. 1994). In eel, PCBs constitute at least 86% of the total TEQ (de Vries 2002).

Normality of data distribution was tested with a Kolmogorov-Smirnov test. For comparison between muscle and gonad samples within groups, one-tailed paired t-tests were performed. For comparison between control and hormone-treated groups, one-tailed univariate analyses of covariance (ANCOVA) was performed. Bodyweight was used as cofactor for differences in fat percentage. Fat percentage was used as cofactor for differences in TEQ level. For correlation analyses, one-tailed Pearson tests were performed for control and hormone-treated groups. Significant correlations with start parameters were analysed with ANCOVA to estimate the determinant. All tests were performed using SPSS 10.0 for Windows. Results were calculated and plotted as means ± SD.

## RESULTS

Control and hormone-treated females were of similar size and age (Table 1), between 6 and 25 years old. Females of the control group had a GSI of 1.4 ± 0.3 (Fig. 1).

Upon hormone-treatment all females fully matured resulting in a significant increase of the GSIs to 27-51 after 12-25 weekly CPE injections (Fig. 1). After stripping the total number of eggs varied between 0.8-1.7 million eggs.

**Table 1** Parameters of artificially matured female silver eels and controls (means ± SD). Eel were treated 12-25 weeks with carp pituitary extracts; condition factor expressed as $W/L^3$; age was estimated by otolithometry; toxicity equivalent to dioxin (TCDD) in ng TEQ/kg fat was determined by the DR-Calux method.

| | | control | hormone-treated |
|---|---|---|---|
| body length (cm) | | 77±8 | 80±5 |
| body weight (g) | | 911±249 | 1095±250 |
| condition factor | | 0.20±0.02 | 0.21±0.02 |
| age (est. years) | | 14±5 | 11±4 |
| TEQ (ng/kg fat) | muscle | 4.9±5.4 | 8.7±7.8 |
| | gonad | 5.6±4.7 | 8.1±7.1 |

Fat percentages in muscle tissue were about 20% both in hormone-treated and control females (range 9 to 35%; Fig. 1). The total fat in gonads of hormone-treated females (58 ± 21 g per kg eel) was 12 times higher than in gonads of control females (5 ± 1 g per kg eel; Fig. 1). Positive correlations with the amount of incorporated fat in the gonads in hormone-treated females were found with age (Pearson; n=13; P=0.012; r=0.619), bodyweight (Pearson; n=13; P=0.026; r=0.549) and condition factor (Pearson; n=13; P=0.006; r=0.668). ANCOVA showed that age (ANCOVA; n=13; P=0.02) was the significant determinant for the amount of incorporated fat. A negative correlation (Pearson; n=13; P=0.036; r=-0.514) was found between age and the amount of fat in muscles in hormone-treated females.

Although the total fat content in the gonads of hormone-treated females was significantly increased (Fig. 1), relative fat levels in the gonads were significantly lower (ANCOVA; n=25; P<0.001). In the mature oocytes of hormone treated females large accumulation of proteic stores (vitellus) had occurred while in the immature oocyte mostly lipid inclusions were found. The TEQ-levels on a fat basis in muscle and gonads tend to be higher in hormone-treated eels (Table 1). TEQ-levels in the muscle were 1.8 times higher, but not significantly different (ANCOVA; n=25; P=0.08). TEQ-levels in the gonads were 1.4 times higher (ANCOVA; n=25; P=0.05) with fat percentage as significant cofactor (ANCOVA; n=25; P=0.008). Average total TEQ-levels in both muscle and gonads of Lake Grevelingen silver eels in this study were slightly lower than the 12 ng TEQ/kg eel measured in eels of the nearby Volkerak in 2001 by van Leeuwen et al. (2002).

During the first hours post fertilisation (hpf), eggs from 8 out of 13 hormone-treated females showed cleavage up to the eight-cell stage. Seven of these 8 batches showed a continued development till 15 hpf but died thereafter. One of these 7 batches resulted in about 1500 embryos at 30 hpf, that however all showed serious oedema of the yolksac; a deformed head region (Fig. 2) and absence of a heartbeat. These embryos died at 43 hpf in

contrast to healthy embryos of another batch, which reached a pre-hatching stage at 100 hpf. A negative correlation (Pearson; n=8; P=0.019; r=-0.736) was found between embryonic survival time from different batches and the corresponding TEQ levels expressed as ng/kg gonad (Fig. 2).

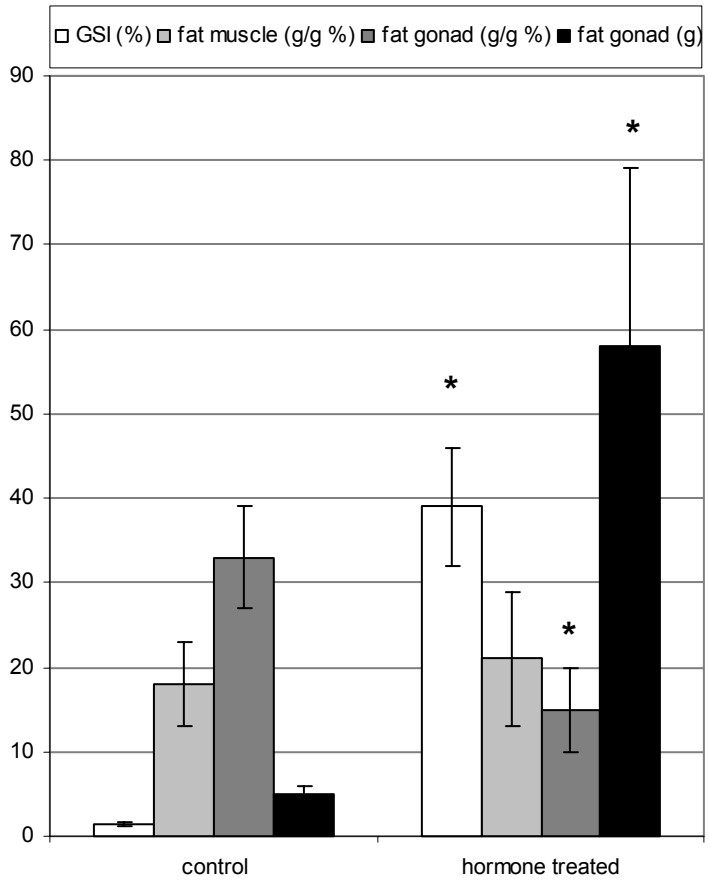


**Figure 1** GSI (relative gonad weight), relative fat content in muscle and gonad (g fat/g tissue *100%) and total fat in gonad (g/kg eel) in control and hormone-treated eels. Highly significant differences (P<0.001) between groups are indicated with an asterisk.

CHAPTER 6

# DISCUSSION

The fat levels in the untreated silver eels in this study are similar to those found in ecological surveys (Svedäng and Wickström 1997). However, the absolute fat content in the gonads was 12 times higher in hormone-treated females showing an extensive fat incorporation in the oocytes. The total fat content of these gonads was between 33 and 103 g per kg eel. For an average 1-kg silver eels with a fat reserve of 200 g this means that 17 to 52% of the total fat reserve is transported into the gonads. The amount of fat transported to the gonads was found positively related to the age of the eel. This suggests an increased capacity of older eels to incorporate more fat from the muscle into the eggs. As egg quality depends heavily on incorporation of reserves, this increased capacity of older eels suggests a higher reproduction potency.

The embryonic malformations observed in our study (Fig. 2) are typical for PCB-exposed eggs such as observed in pike *Esox lucius* (Helder 1980), carp *Cyprinus carpio* (Stouthart et al. 1998), lake trout *Salvelinus namaycush* (Walker et al. 1994) and rainbow trout *Oncorhynchus mykiss* (Walker and Peterson 1991). Similar symptoms are described for fish-eating birds and are known as GLEMEDS or 'Great Lakes embryo mortality, edema and deformities syndrome' (Gilbertson et al. 1991). The observed correlation between embryo survival time and TEQ levels in the gonads implies TEQ-induced teratogenic effects. The disrupting effects occurred at levels below 4 ng TEQ/kg gonad, below the EU eel consumption standard. Since July 2002 this is set at 4 ng TEQ/kg filet, thus far based only on dioxins and furanes (Anonymous 2001). Total TEQ levels in wild eels from all Dutch locations are in the range between 1 and 61 ng TEQ/kg eel. In the same study TEQ levels from 10 locations in 5 other European countries were found between 0 and 20 ng TEQ/kg eel (van Leeuwen et al. 2002). Only eels from Sardinia (Italy) and some locations in Ireland had TEQ levels below the limit of detection. Therefore most of the TEQ-levels in wild eel are above the levels severely impairing recruitment in our study.

In addition, migrating silver eels will use at least 60g fat/kg eel (40% of the total fat reserves) for their spawning migration (van Ginneken and van den Thillart 2000). This means, considering a biological half-live of PCBs between 1 to 4 years (de Boer et al. 1994), an increase in the concentration of the dioxin-like compounds with at least 40%. So, the TEQ values in gonads of the eels spawning in the Sargasso Sea will be even higher than those in the gonads of the artificially spawned eels in this study.

Our study suggests that current gonadal levels of dioxin-like contaminants, including PCBs, in eels from most European locations impair normal embryonic development. This conclusion is further strengthened by the fact that the emission of PCBs in the environment (van Leeuwen and Hermens 1995) coincides with the decline of eel populations (Anonymous 2003). Therefore we consider it likely that dioxin-like PCBs contributed to the current collapse of the European eel populations.

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CHAPTER 7

Otolith of a 26-year old eel. Age is a determinant for successful eel reproduction since: a) older eels were more susceptible to swimming-induced oocyte development; b) older eels showed increased capacity to incorporate more fat from the muscle into the oocytes determining higher egg quality; and c) older eels were more sensitive to hormonal stimulation.

# Chapter 7

**The fate of fat in silver eels: lipid requirements for spawning migration**

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Keywords: European eel, *Anguilla anguilla*, swimming, cost of transport, endurance, energy, efficiency, oxygen consumption, maturation, reproduction

This chapter will be submitted to Marine Ecology Progress Series

CHAPTER 7

ABSTRACT
The energy budget of semelparous eels is a true example of biological efficiency. They metamorphose (silvering) preparing for their 5,500-km oceanic reproductive migration to the Sargasso. Since they cease feeding, their stored energy, mainly as lipids in muscle and under the skin, should suffice for both migration and incorporation in the oocytes. Few attempts however were made to estimate the energy costs. Recently, we found that optimum swim speeds of silver eels are around 0.8 BL/s, higher than the generally assumed cruise speeds of 0.5 BL/s. At those speeds, large silver eels may reach the spawning grounds in about 105 days. In this study we therefore subjected farmed eels and wild large silver eels to simulated migration at those speeds and calculated cost of transport (COT) from oxygen consumption ($\dot{M}O_2$) rates. We found that farmed eels swam at COTs of 34 ± 5 mg $O_2$/kg/km during 2,173 ± 305 km migration, while wild silver eels swam at higher COTs of 52 ± 12 mg $O_2$/kg/km during 1,232 ± 172 km migration. COTs were rather constant and similar to values obtained from short term 2h swim tests. Wild silver eels spend 78 ± 4 g fat /kg on a complete 5,500-km migration run. These relatively low values are proof of a high metabolic efficiency. We artificially matured eels from the same batch of wild silver eels by hormonal injections to determine the amount of fat that was incorporated in the oocytes. We found that eels incorporate 57 ± 22 g fat /kg in the oocytes during artificial maturation. The amount of fat transported to the gonads was found to be positively related to the age of the eel. In total, European eels may therefore spend about 135 g fat/kg on their spawning run. Fat stores of silver eels from high quality trophic habitats should suffice for successful reproduction.

## INTRODUCTION

A true example of biological efficiency concerns the energy budget of eels for reproduction. All eel species exhibit an impressive reproductive migration of which the European eels swim the longest distance i.e. some 5,500-km to the assumed spawning grounds in the Sargasso Sea (Schmidt, 1923; Tesch 2003). They spend their feeding stage as immature yellow eels in the fresh and brackish European waters. At the end of each growth season, some eels cease feeding and metamorphose (silvering) in preparation of their oceanic journey. Probably their fat content plays a major role in the onset of silvering and their seaward migration (Larsson et al., 1990; Svedäng & Wickström, 1997). Their stored energy, mainly as lipids in muscle and under the skin, should suffice for two major purposes: migration and reproduction.

Eels are believed to exhibit a semelparous strategy meaning that individuals spawn only once in their lifetime. Semelparous spawners exceed a boundary to survive and they die shortly after reproducing. The boundary between semelparous and iteroparous lifestyles lies within a 60-70% energy depletion range (Wootton, 1990). Iteroparous trouts spend 40-50% energy on spawning with 3-4% of gonadal energy (Jonsson, 2005). Semelparous Pacific salmons (*Oncorhynchus* spp.) spend 75-82% with 10% of gonadal energy (reviewed by Lucas & Baras, 2001). A legitimate estimation of the cost of reproduction of eel is still lacking.

In his 'new solution to the Atlantic eel problem', Tucker (1959) suggested, that the European eel could never reach the *Anguilla* breeding area in the Sargasso. Since then, few attempts were made to estimate the energy costs of migration and maturation. Simulated migration trials that were recently carried out by our group provided new insight. They were performed in 22 Blazka-type 127-L swimtunnels in which the oxygen levels were measured continuously by oxygen electrodes (Van den Thillart et al., 2004). Van Ginneken & Van den Thillart (2000) extrapolated results of a 387 km swim trial and estimated that eels swim at 0.573 kJ/kg/km, a cost of transport (COT) 2.4-3.0 times lower than that calculated by Schmidt-Nielsen (1972). Van Ginneken et al. (2005b) calculated energy consumption of a full 5,500-km simulated migration by both oxygen consumption ($\dot{M}O_2$) and bomb-calorimetry. They came to the same conclusion and found that eels spent only 0.418-0.611 kJ/kg/km. Van den Thillart et al. (2004) extrapolated results of a 2,850-km swim trial and found slightly higher values of 0.833 kJ/kg/km. The latter two long distance experiments were performed with 3 year old farmed eels swimming in fresh water at a speed of 0.5 boy length per second (BL/s), the presumed cruise speed to the Sargasso (Tesch, 2003). This might, however, not be a correct representative for the natural situation. Palstra et al. (*chapter 2*) found optimum swim speeds for farmed eels in fresh water of 0.96 BL/s and for wild eels from Lake Grevelingen in salt water lower, of 0.77 BL/s. Although COT was rather constant at all swim speeds, it was the lowest at swim speeds around 0.8 BL/s. Therefore it was assumed that this speed may represent the actual cruise speed to the Sargasso. These speeds of 0.8 BL/s are much higher than the cruise speeds that were presumed until now (Tesch, 2003). The natural situation differs however in more perspectives. Wild silver eels have for instance a lower condition factor and lower fat percentages than farmed eels. Also is migration in reality performed in salt water and at lower temperatures. Palstra et al. (*chapter 2*) found that energy costs of swimming are 20% higher in SW in comparison to FW.

Besides the costs of migration, the development of the gonads up to gonadosomatic indices (GSIs) of 28 to 60 (*chapter 5*) requires a substantial part of the energy reserves. Boëtius & Boëtius (1980) measured lipid and protein contents in the gonads of two strip-ripe eels. Eel 1 had an initial body weight of 940 g, a gonad weight of 467 g (GSI=46.8) of which 43.4 g was fat and 36.9 g protein. Eel 2 had an initial body weight of 780 g, a gonad weight of 442 g (GSI=45.5) of which 23.4 g was fat and 29.2 g protein. By using conversion factors of 38.9 kJ/g for lipids and 17.2 kJ/g for protein, they calculated that 1.41 and 2.32 MJ was utilised for gonadal development. Until now this is the only calculation available.

In order to establish a legitimate estimation of the total cost of eel reproduction, we need 1) to determine the costs of transport at higher, near optimal, swim speeds, 2) to mimic natural migration conditions e.g. subjecting wild silver eels to swimming in SW at low temperature and 3) to determine the energy costs of gonad development of wild silver eels of the same batch

Therefore, two kinds of experiments were performed in this study: simulated migration and artificial maturation by hormonal stimulation. To investigate the effects of higher swim speeds during long-term swimming (1), we subjected 5 year-old farmed female eels to a swim trial in fresh tap-water (FW at 18°C) at speeds of 0.8 BL/s. We expect lower COTs at this speed in comparison with the generally applied 0.5 BL/s. To

mimic natural conditions (2), a similar swim trial was performed with wild female silver eels from Lake Grevelingen but in artificial sea water (SW) at low temperature (from 18°C down to 10°C). Swimming in SW results in a 20% higher COT (*chapter 2*). Like with speed, a temperature exists at which $\dot{M}O_2$ and swim performance are optimal (reviewed by Beamish, 1983) but which is, however, unknown for eel. To determine the energy costs of gonad development of these eels (3), fat contents in muscle and gonad tissue were determined in artificially matured eels. The total cost of eel reproduction was established as the sum of the cost of migration and the cost of gonad development.

## MATERIAL AND METHODS

Formulas used for calculation of different parameters are given in table 1.

**Table 1** Formulas used for calculation of parameters.

**Formulas for sampling parameters:**

1. $\dot{M}O_2 = 127* \Delta[O_2]/\Delta t$ ($mgO_2/kg/h$), where: $\Delta[O_2]/\Delta t$ is the decrease of the oxygen content per hour
2. $K= 100* BW/BL^3$
3. $EI= 100* ((EDh+EDv)/4)^2\pi/10*BL)$
4. $PFLI= 100* PFL/BL$
5. GSI= (Weight gonads / Body weight) *100%

**Formulas for calculation of cost of transport (COT) from weight loss:**

6. $COT_{tot}$ (kJ/kg/km)= $E_{fat} + E_{protein}+ E_{carbohydrate}$,
7. $dBW_{loss} = 0.50* wBW_{loss}$
8. $COT_{fat}$ (g fat/kg/km)= $0.68* dBW_{loss}$
9. $COT_{fat}$ (kJ/kg/km)= $39.5* COT_{fat}$ (g fat/kg/km)
10. $COT_{protein}$ (g protein/kg/km)= $0.28* dBW_{loss}$
11. $COT_{fat}$ (kJ/kg/km)= $23.6* COT_{fat}$ (g fat/kg/km)
12. $COT_{carbohydrate}$ (g carbohydrate/kg/km)= $0.01* dBW_{loss}$
13. $COT_{carbohydrate}$ (kJ/kg/km)= $17.2* COT_{carbohydrate}$ (g carbohydrate/kg/km)

**Formulas for calculation of cost of transport (COT) from oxygen consumption:**

14. $COT_{tot}$ (kJ/kg/km)= $COT_{fat} + COT_{protein}+ COT_{carbohydrate}$
15. $COT_{fat}$ (kJ/kg/km)= $13.72*(0.798*COT$ (g $O_2$/kg/km))
16. $COT_{fat}$ (g fat/kg/km)= $(1/39.5) * COT_{fat}$ (kJ/kg/km)
17. $COT_{protein}$ (kJ/kg/km)= $13.36*(0.196*COT$ (g $O_2$/kg/km))
18. $COT_{protein}$ (g protein/kg/km)= $(1/23.6) * COT_{protein}$ (kJ/kg/km)
19. $COT_{carbohydrate}$ (kJ/kg/km)= $14.76*(0.005*COT$ (g $O_2$/kg/km))
20. $COT_{carbohydrate}$ (g carbohydrate/kg/km)= $(1/17.2) * COT_{carbohydrate}$ (kJ/kg/km)

*Experimental eels*

Five year-old female eels (600-1,400 g; 60-90 cm; n=12) were obtained from a commercial hatchery (Royaal BV Helmond, The Netherlands; Table 2). Silver female eels (500-1,700 g; 60-90 cm; n=31) were caught in the fall of 2001, 2002 and 2003, during their seaward migration in the brackish Lake Grevelingen (Bout, Bruinisse, The Netherlands) at the North Sea sluice at 32 ppt. After arrival in the lab, all eels were anaesthetized with oil of cloves (1:10 dissolved in 100% ethanol using a dosage of 1.5 ml/l water). Six farmed eels and six eels from Lake Grevelingen were killed and sampled as untreated control groups. Other eels were tagged with small passive transponders (TROVAN, EID Aalten BV, Aalten, The Netherlands).

*Experimental swim-tunnel set-up*

A set of 22 Blazka-type 127-L swimtunnels as described by Van den Thillart et al. (2004) was used for the swim trials. The swim tunnels were placed in the direction of the Sargasso Sea (WNW) in a climatized room of about 100-$m^2$. The total water content of the system was about 7000-L and was recirculated continuously over a bio-filter, a sand-filter, and UV-lamps. The illumination in the climatized room was switched to 670-nm light (bandwidth 20-nm). Based on pigment changes during silvering, it is very unlikely that this far-red light is invisible for eels (Pankhurst & Lythgoe 1983). The oxygen level in all swim tunnels was measured continuously by oxygen electrodes (Mettler Toledo). The latter were calibrated with sodium-sulfite (0%) and air (100%). When the oxygen levels came below 75% air saturation (AS), the water refreshment was switched on by the controller, automatically raising it up to 85% AS. The $\dot{M}O_2$ rate was calculated from the oxygen decline after automatic closure of the water-inlet by a magnetic valve. From the decline of the $O_2$-concentration, the $\dot{M}O_2$ rate was calculated following formula (1). The body weight (BW) of each eel was calculated for each day of migration from a linear relation between BW before and after the trials.

*Protocol experiment 1: swim trial with 5 year old farmed eels in fresh water*

In May 2003, 6 eels were anaesthetized and morphometric parameters were measured. They were introduced in the swim tunnels in running fresh water (FW) of 18 °C. Swimming was started after two days of rest. During the first 4 days, the swim speed was increased from 0.5 to 0.8 BL/s with increments of 0.1 BL/s per day. The eels were kept at 0.8 BL/s for 27 days followed by 17 days at 0.7 BL/s. After this swim period, eels were removed from the tunnels, anaesthesized, measured, killed and sampled.

*Protocol experiment 2: swim trial with Lake Grevelingen eels in salt water*

In January 2003, 6 eels were anaesthetized and morphometric parameters were measured upon arrival from Lake Grevelingen. Thereafter, they were introduced in the swim tunnels in salt water (SW; 32 ppt) at 18 °C. After two days of rest, they started to swim at 0.5 BL/s and swim speed was increased like described above. Starting at day 4, water temperature was lowered with 0.5 ºC per day from 18 ºC down to 10 ºC. This temperature was kept stabile during further swimming. The experiment was stopped after 26 days, the eels were immediately removed from the tunnels, anaesthetized, measured, killed and sampled.

*Protocol Experiment 3: Artificial maturation of Lake Grevelingen eels*

Thirteen Lake Grevelingen eels were anaesthetized and morphometric parameters were measured before they were treated with carp pituitary extract (*chapter 5* and *6*). They were administered weekly injections of Carp Pituitary Extract (CPE: 20-mg/kg) until oocytes were displaying germinal vescicle breakdown and ovulation could be induced with 17,20β-dihydroxy-4-pregnen-3-one (DHP: 2-mg/kg), and they could be hand-stripped (*see for detailed description chapter 5*).

*Morphometric measurements and sampling*

Morphometric parameters included bodylength (BL), bodyweight (BW), eye diameters horizontal and vertical (EDh, EDv) and pectoral fin length (PFL). Eels (control, swim and hormone treated groups) were sampled for gonad tissue. With these measurements we determined:

- Fulton's condition factor K using formula (2)
- The eye index according to Pankhurst (1982) EI using formula (3)
- The pectoral fin length index according to Durif et al. (2005) PFLI using formula (4)
- The silver index (SI) according to Durif et al. (2005) based on BL, BW, ED and PFL.
- Gonadosomatic index (GSI) using formula (5)

Otolithes (sagitta) were removed for age determination according to the method by Daverat (2005a) as described in *chapter 2*. From artificially matured eels and their controls, a gonad tissue sample (at a standardised rostral location) and a muscle tissue sample (2x2x1cm, taken dorsal from the lateral line at the position of the genital pore) were stored at -20° for fat measurement.

*Fat measurement*

Portions of 1 g were homogenized in 2 ml ultra pure water. Isopropanol (2 ml) was added, mixed and subsequently fat was extracted using 3 portions of 4 ml hexane/di-ethyl-ether (97:3). After evaporation of the solvent, fat was determined gravimetrically.

*Statistics*

Normality of the data distribution was tested with Kolmogorov-Smirnov tests. Paired t-tests with one-tailed probabilities were used for differences of parameters body weight (BW), condition factor (K), eye index (EI), pectoral fin index (PFI) comparing pre- and post swimming or pre- and post hormonal treatment. A Wilcoxon test with one-tailed probabilities was used to test for differences in pre- and post silver index (SI). Difference in BW between the farmed and Lake Grevelingen eels was tested with a unpaired t-test with two-tailed probabilities.

In order to test for a relation between water temperature and $\dot{M}O_2$, a Pearson correlation test with one-tailed probabilities was performed for mean datapoints during the trajectory of water temperature decrease from 18 to 10 °C during simulated migration of Lake Grevelingen eels. To test for energy differences of farmed eels swimming in FW vs. Lake Grevelingen eels swimming in SW, univariate analyses of variance (ANCOVA) with

co-factor BW was performed on oxygen consumption ($\dot{M}O_2$) and weight loss ($BW_{loss}$) data. A Pearson correlation test with one-tailed probabilities was used to test for correlation between $\dot{M}O_2$ and BW loss. For comparison between muscle and egg samples within groups, paired one-sided t-tests were performed. For comparison between control and treated groups, ANCOVA was performed. Pearson correlation with one-tailed probabilities between the start parameters age, body length (BL), BW vs. swim parameters ($\dot{M}O_2$, $BW_{loss}$ and distance) and vs. maturation parameters (no inj, GSI, fat percentage muscle, fat percentage gonads, total fat gonads) was tested for predictive significance. Significant correlations were analysed with ANOVA to estimate the determinant. All tests were performed in SPSS 10.0 for Windows. Results were calculated and plotted as means ± SD.

## RESULTS

### *1 Migration*

#### Experiment 1: swim trial with 5 year old farmed eels in FW

*Oxygen consumption and weight loss during migration of farmed eels*

Mean $\dot{M}O_2$ rates peaked at day 5, a day after the last speed increment (Fig. 1). After this peak, during the period of swimming at 0.8 BL/s (day 6-31), values became fairly stable at a mean of 75 ± 11 mg $O_2$/kg/h corresponding to a COT of 34 ± 5 mg $O_2$/kg/km (Table 2). From day 31 to 45, eels swam at 0.7 BL/s. After 45 days of swimming, the eels were stopped at a mean distance of 2,173 ± 305-km (range 1,717-2,447-km). During this period they lost on average 64 ± 32 g corresponding to 30 ± 15 mg/kg/km (Table 2).

*Morphometric changes during simulated migration of farmed eels*

In table 3a an overview is given of external parameters before and after swimming. Eels were all 5 years old, 76 ± 4 cm long and weighted 951 ± 95 g at the start. They were silver as indicated both by the eye index (11.2 ± 1.9) and silver index (3.83 ± 0.41). After swimming, the eyes had increased significantly ($P<0.01$)! The eye index increased with 8% to 12.1 ± 1.8. However, the GSI did not differ from the control group.

#### Experiment 2: swim trial with Lake Grevelingen eels in SW

*Oxygen consumption and weight loss during migration of Lake Grevelingen eels*

The mean $\dot{M}O_2$ of the six swimmers is plotted per day in Fig. 2. During the first four days the speed is raised from 0.5 up to 0.8 BL/s which correlates with a rise in the $\dot{M}O_2$. After reaching a speed of 0.8 BL/s at day four, and during decrease of the water temperature from 18 to 10°C, the $\dot{M}O_2$ rates remained stable at an mean of 108 ± 5 mg/kg/h corresponding to a COT of 52 ± 12 mg $O_2$/kg/km (Table 2). No significant correlation was found between water temperature and $\dot{M}O_2$ rates. After 26 days of swimming, the eels were stopped at an mean distance of 1,232 ± 171 km (range 909-1361

km). During this period they lost on average 48 ± 19 g corresponding to 54 ± 30 mg /kg/km (Table 2).

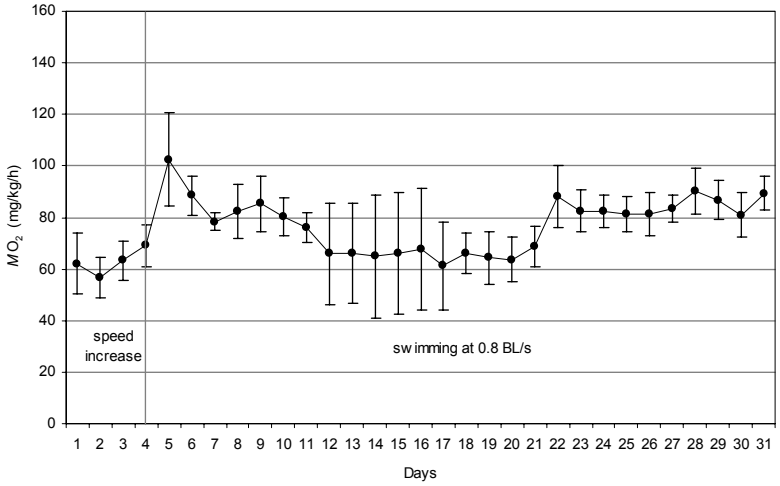


**Figure 1** Daily mean of oxygen consumption rate ($\dot{M}O_2$) of farmed eels (n=6) in 18 °C fresh water (FW) swimming 27 days at 0.8 BL/s. The swim speed was increased the first four days from 0.5 bodylength per second (BL/s) to 0.8 BL/s with increments of 0.1 BL/s per day.

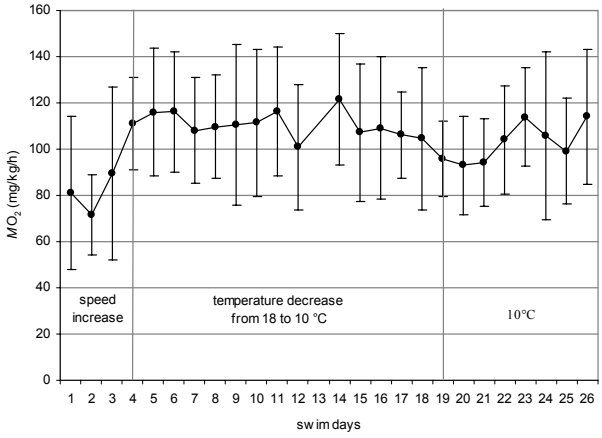


**Figure 2** Mean $\dot{M}O_2$ of Lake Grevelingen eels (n=6) in salt water (SW) swimming 26 days (1,232 ± 171 km). Speed was increased the first four days from 0.5 BL/s to 0.8 BL/s with increments of 0.1 BL/s per day. Starting at day 4, the water temperature was lowered with 0.5 ºC per day from 18 ºC to 10 ºC.

**Table 2** Individual values for parameters during 2 swim experiments: farmed eels in fresh water (FW) or Lake Grevelingen eels in salt water (SW). Data represent: their age in years; body length (BL) in cm; swim distance in km; BW in g before and after swimming; the body weight loss ($BW_{loss}$); oxygen consumption rate ($\dot{M}O_2$) and cost of transport (COT). Asterisks mark values of eels that contained much fluid in intestine and bladder after swimming resulting in higher BW.

| | | | | | pre-swimming | post swimming | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | age | BL | swim distance | BW | BW | $BW_{loss}$ | | | $\dot{M}O_2$ | COT |
| group | no | (years) | (cm) | (km) | (g) | (g) | (g) | (g/kg) | (mg/kg/km) | (mg $O_2$/kg/h) | (mg $O_2$/kg/km) |
| Cultured eels | 1 | 5 | 69 | 1717 | 883 | 877 | 6* | 7 | 4 | 70 ± 11 | 35 ± 5 |
| (FW, 18 °C) | 2 | 5 | 75 | 2380 | 964 | 895 | 68 | 71 | 30 | 74 ± 11 | 34 ± 5 |
| | 3 | 5 | 78 | 2447 | 908 | 812 | 96 | 105 | 43 | 83 ± 9 | 37 ± 4 |
| | 4 | 5 | 81 | 1890 | 1127 | 1069 | 59 | 52 | 28 | 56 ± 25 | 24 ± 11 |
| | 5 | 5 | 76 | 2189 | 865 | 777 | 88 | 101 | 46 | 86 ± 13 | 39 ± 6 |
| | 6 | 5 | 78 | 2416 | 957 | 891 | 66 | 69 | 29 | 79 ± 11 | 35 ± 5 |
| | **av** | **5** | **76** | **2173** | **951** | **887** | **64** | **68** | **30** | **75** | **34** |
| | **stdev** | **0** | **4** | **305** | **95** | **101** | **32** | **36** | **15** | **11** | **5** |
| Lake Grevelingen eels SW | 1 | 7 | 71 | 1247 | 787 | 773 | 15* | 19 | 15 | 82 ± 10 | 40 ± 5 |
| (SW, 18 to 10 °C) | 2 | 13 | 76 | 1343 | 852 | 787 | 65 | 77 | 57 | 130 ± 19 | 59 ± 9 |
| | 3 | 8 | 72 | 909 | 683 | 616 | 66 | 97 | 107 | 147 ± 15 | 71 ± 7 |
| | 4 | 9 | 68 | 1194 | 589 | 551 | 38 | 64 | 54 | 109 ± 13 | 55 ± 6 |
| | 5 | 6 | 78 | 1361 | 901 | 852 | 49 | 55 | 40 | 105 ± 11 | 47 ± 5 |
| | 6 | 7 | 76 | 1336 | 759 | 708 | 51 | 68 | 51 | 87 ± 9 | 40 ± 4 |
| | **av** | **8** | **73** | **1232** | **762** | **714** | **48** | **63** | **54** | **108** | **52** |
| | **stdev** | **2** | **4** | **171** | **113** | **113** | **19** | **26** | **30** | **5** | **12** |

**Table 3** Values of morphometric parameters for eels before (pre) and after (post) the different swim experiments; a) simulated migration (2,173 ± 305 km) of farmed eels in FW, and b) simulated migration (1,232 ± 171 km) of Lake Grevelingen eels in SW. In bold are given significant differences (P<0.01) between pre and post measurements. GSI values before experiments (*italics*) are given from a control group that was sampled upon arrival in the lab.

| | a) **Experiment 1 simulated migration farmed eels FW (n=6)** | | | | b) **Experiment 2 simulated migration Lake Grevelingen eels SW (n=6)** | | | |
|---|---|---|---|---|---|---|---|---|
| | pre | | post | | pre | | post | |
| **parameter** | mean | SD | mean | SD | mean | SD | mean | SD |
| age (years) | | | 5 | 0 | | | 8 | 2 |
| bodylength (cm) | 76 | 4 | 76 | 4 | 73 | 4 | 73 | 4 |
| bodyweight (g) | 951 | 95 | **887** | **101** | 762 | 113 | **714** | **113** |
| condition factor | 0.22 | 0.03 | **0.19** | **0.04** | 0.19 | 0.02 | **0.18** | **0.02** |
| ocular index | 11.2 | 1.9 | **12.1** | **1.8** | 11.5 | 1.6 | 11.8 | 1.7 |
| pectoral fin index | 3.9 | 0.25 | 3.85 | 0.24 | 4.62 | 0.31 | 4.60 | 0.21 |
| silver index | 3.83 | 0.41 | 4 | 0.63 | 4.17 | 0.75 | 4.33 | 0.82 |
| gonadosomatic index | *1.43* | *0.23* | 1.19 | 0.09 | *1.13* | *0.18* | 1.24 | 0.26 |

**Table 4** Values of morphometric parameters for eels before (pre) and after (post) inducing maturation by 17 ± 4 CPE injections of Lake Grevelingen eels. In bold are given significant differences (P<0.01) between pre and post measurements. GSI values before experiments (*italics*) are given from the control group that was sampled upon arrival in the lab.

| | **Experiment 3 artificial maturation Lake Grevelingen eels (n=13)** | | | |
|---|---|---|---|---|
| | pre | | post | |
| **parameter** | mean | SD | mean | SD |
| age (years) | | | 11 | 4 |
| bodylength (cm) | 81 | 6 | 81 | 6 |
| bodyweight (g) | 1131 | 297 | **1256*** | **291** |
| condition factor | 0.21 | 0.02 | **0.23** | **0.03** |
| ocular index | 11.7 | 1.9 | **15.4** | **2.2** |
| pectoral fin index | 4.81 | 0.25 | 5.16 | 1.04 |
| silver index | 3.90 | 0.32 | 4.10 | 0.32 |
| gonadosomatic index | *1.13* | *0.18* | 37.1 | 9.4 |

* weight taken at the moment of DHP injection

*Morphometric changes during simulated migration of Lake Grevelingen eels*

Eels were on mean 8 ± 2 years old (range 6-13; table 3b). They were 73 ± 4 cm long and weighed 762 ± 113 g at the start. They were silver as indicated both by the eye index (11.5 ± 1.6) and silver index (4.17 ± 0.75). After swimming, eye or silver indices had not increased significantly. Also the GSI did not differ from the control group.

## *2 Maturation*

*External changes after artificial maturation*

Hormone-treated eels were on mean 11 ± 4 years old (range 6-20; Table 4). They were 81 ± 6 cm long and weighed 1,131 ± 297 g at the start. Upon hormone-treatment all females fully matured resulting in a significant increase of the GSIs to 37 ± 9% after 17 ± 4

weekly CPE injections. Other significant changes that indicated maturation were increases in body weight (11%, P<0.001; Table 2) and condition factor (10%, P<0.001). The eye index also increased significantly (32%, P<0.001; Table 2) from 11.7 ± 1.9 to 15.4 ± 2.2. Other signs of silvering like pectoral fin index and the silver index increased but not significantly.

*Fat incorporated in oocytes*

Data on Lake Grevelingen eels as presented here have already been reported together with data of 7 eels from River Loire (France) in *chapter 6*. Control eels had a GSI of 1.13 ± 0.18% (Fig. 3a). When having reached a mean GSI of 37 ± 9% after treatment (Fig. 3a), the total fat in the gonads of the hormone-treated females was 57 ± 22 g per kg eel (Fig. 3b), 12 times higher than in control eels (4 ± 1 g per kg eel). Fat percentages in muscle tissue of control eels were 21 ± 8% (range 11 to 35%; Fig. 3c) and remained similar after treatment (20 ± 5%). Fat percentages in the gonad tissue of control eels were 33 ± 5% (Fig. 3d) and significantly lower (P≤0.01) after treatment. Significant differences (P≤0.01) existed between fat in muscle and gonad between control and treated eels

*Correlations between status at the start and maturation performance*

Correlation analysis showed a significantly (P<0.05) negative correlation between age and the number of injections needed to mature the females (Table 4). Furthermore age showed a negative correlation with muscle fat percentage (P<0.05) and positive with gonad fat percentage (P<0.05) and with total fat in the gonads (P<0.01). ANCOVA showed that age (P=0.02) was the significant determinant for the amount of incorporated fat. BL and BW showed a negative correlation (P<0.01) with GSI.

**Table 5** Pearson correlations between parameters age, BL and BW at the start of hormonal injections and parameters at full maturation; the required number of CPE injections, GSI, fat percentage in muscle and gonads, and total fat in the gonads. Shown are the correlation, the P-value and the number of observations n. In bold, significant correlations.

| | | **end parameters:** | | | | |
|---|---|---|---|---|---|---|
| **start parameters:** | | **no inj** | **GSI** | **fat muscle (%)** | **fat gonad (%)** | **total fat gonad** |
| **age** | corr. | **-0.495** | 0.069 | **-0.519** | **0.514** | **0.743** |
| | P | **0.043** | 0.411 | **0.035** | **0.036** | **0.002** |
| | n | **13** | 13 | **13** | **13** | **13** |
| **BL** | corr. | -0.345 | **-0.719** | -0.472 | 0.405 | 0.163 |
| | P | 0.12 | **0.003** | 0.052 | 0.085 | 0.298 |
| | n | 13 | **13** | 13 | 13 | 13 |
| **BW** | corr. | -0.228 | **-0.713** | -0.455 | **0.63** | 0.275 |
| | P | 0.226 | **0.003** | 0.059 | **0.011** | 0.182 |
| | n | 13 | **13** | 13 | **13** | 13 |

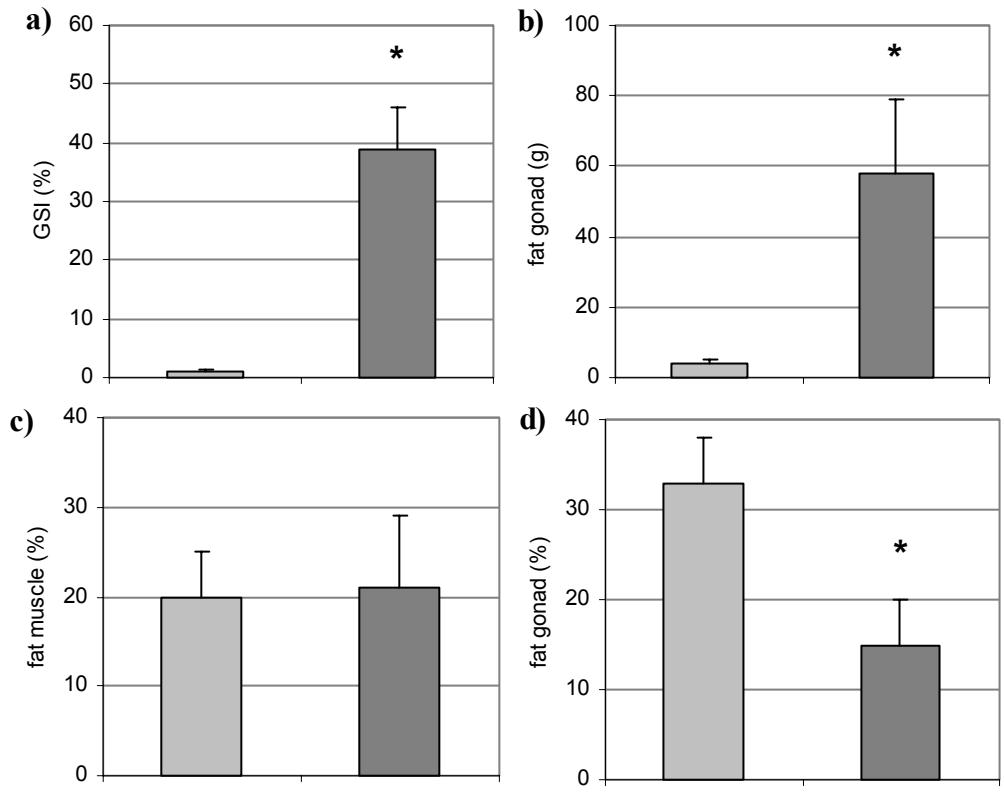


**Figure 3** a) GSI (relative gonad weight), b) total fat in gonad (g/kg eel) in control (light grey) and hormone-treated (dark grey) eels, c) relative fat content in muscle and d) gonad (g fat/g tissue *100%). Significant differences (P≤0.01) are indicated by the asterisks.

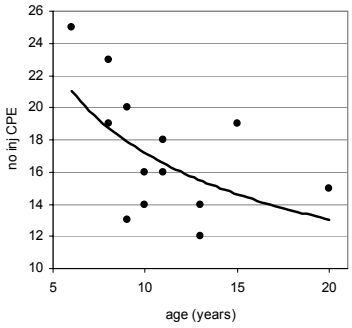


**Figure 4** Significant relation (Pearson correlation P<0.05, ANCOVA P=0.02) between age and the number of injections CPE required to fully mature Lake Grevelingen silver eels.

## DISCUSSION

*Effect of swim speed*

Farmed eels in FW swam for 28 days at 0.8 BL/s at oxygen consumption ($\dot{M}O_2$) values of 75 ± 11 mg/kg/h. The cost of transport (COT) was found 34 ± 5 mg/kg/km. These values were similar to the values observed during swim fitness tests (*chapter 2*). During this test, eels swam for just 2 h per speed. At a slightly higher speed of 0.85 ± 0.05 BL/s, the $\dot{M}O_2$ values were 82 ± 12 mg/kg/h and COT values were 39 ± 5 mg/kg/km. Van den Thillart et al. (2004) and Van Ginneken et al. (2005b) used similar sized farmed eels swimming in FW at 0.5 BL/s and found $\dot{M}O_2$ levels of resp. 37 ± 3 and 42 ± 6 mg/kg/h. COT values were resp. 28 ± 2 and 32 ± 3 mg/kg/km. This indicates that COT values remain similar when the swim speed is increased with 60% from 0.5 BL/s to 0.8 BL/s. This result agrees with the conclusion from swim fitness tests (*chapter 2*) that COT is very low and rather constant at all swim speeds. Eels are therefore excellent cruisers that, in absence of a strictly defined optimum swim speed, may easily alter the cruise speed of preference in a range of 0.5 to 1.0 BL/s.

*Effect of salinity and water temperature*

Farmed eels and Lake Grevelingen eels were of similar length (Table 3ab), which makes comparison of swim performance legitimate. However, at the same length farmed eels were significantly heavier (P=0.01), having significantly higher K and having, in contrast to the Lake Grevelingen eels, large amounts of intestinal fat. Both groups were also comparable with respect to their silver index. All experimental eels were silver eels (EI>6.5) and, except for one farmed and one Grevelingen eel, all were in a migratory phase (stage 4 or 5). Lake Grevelingen eels swimming in SW exhibited significantly (P<0.01) higher $\dot{M}O_2$ values (44%) and COTs (53%) than the farmed eels swimming in FW. Also in the former experiments such differences were found in $\dot{M}O_2$ values and COTs between farmed eels in FW with Lake Grevelingen eels in SW. Van den Thillart et al. (2004) and Van Ginneken et al. (2005b) used farmed eels in FW and found $\dot{M}O_2$ values of resp. 37 ± 3 and 42 ± 6 mg/kg/h, and COT values of 28 ± 2 and 32 ± 3 mg/kg/km. Van Ginneken & Van den Thillart (2000) used Lake Grevelingen eels in SW and found $\dot{M}O_2$ values of 66 ± 14 mg/kg/h, and COT values of 42 ± 10 mg/kg/km. Thus, in these studies $\dot{M}O_2$ values were at least 57% higher and the COT values were 31% higher of Lake Grevelingen eels in SW vs. farmed eels in FW. Comparing $\dot{M}O_2$ values of similar farmed eels in either FW or SW during swim fitness tests (*chapter 2*) showed a difference in COT of only 20%. Paired observations of the Lake Grevelingen eels showed no significant change in $\dot{M}O_2$ during the 8°C decrease, suggesting that energy costs of eel swimming is indepent from water temperature. So, the additional 33% increase in COT cannot be ascribed to differences in either salinity or water temperature and thus reflects a lower condition of wild Lake Grevelingen eels in comparison with farmed eels.

**Table 6** Comparison of estimated energy costs for migration (COT) and maturation (COM) of eel in literature and this study with a) reference, experiment, conditions, and migration costs in kJ/kg eel/km ($COT_{tot}$) and required fat in g fat/kg eel/km, and b) maturation costs in required fat.

Energy cost of transport (COT)

| experiment | conditions | $COT_{tot}$ | $COT_{fat}$ | ref |
|---|---|---|---|---|
| | | kJ/kg/km | mg fat/kg/km | |
| <1day | male (?) yellow and silver (250g)<br>FW, 0.35-0.65 m/s, 15 °C | 1.37-1.74 | 27.8-35.3 | 1 |
| 387 km | 5 female silver eels (± 1 m)<br>SW, 0.5 BL/s, 14 °C | 0.57 | 11.5 | 2 |
| 2,850-km | 5 female farmed eels (919 g)<br>FW, 0.5 BL/s, 19 °C | 0.833 | 16.7 | 3 |
| 5,533-km | 9 female farmed eels (3 years old, 915 g)<br>FW, 0.5 BL/s, 19 °C | 0.418/0.611* | 8.4/12.4* | 4 |
| 2,173-km | 6 female farmed silver eels (5 years old, 951 g)<br>FW, 0.8 BL/s, 18 °C | 0.469/0.522* | 9.5/10.5* | 5 |
| 1,232-km | 6 female Lake Grevelingen silver eels (6-13 years old, 762 g)<br>SW, 0.8 BL/s, 10-18 °C | 0.702/0.860* | 14.2/17.5* | 5 |

* measured by two methods: resp. oxygen consumption and carcass analysis (van Ginneken et al., 2005) or weight loss (this study)

ref 1 Schmidt-Nielsen (1972)
2 Van Ginneken & Van den Thillart (2000)
3 Van den Thillart et al. (2004)
4 Van Ginneken et al. (2005b)
5 this study

Energy cost of maturation (COM)

| conditions | $COM_{fat}$ | ref |
|---|---|---|
| | g fat/kg | |
| 2 female silver eels (960 and 780 g)<br>SW, 22-25 °C | 29/46 | 1 |
| 13 female Lake Grevelingen silver eels (6-20 years old, 1131 g)<br>SW, 18 °C | 57 | 2 |

ref 1 Boetius & Boetius (1980)
2 this study

*Cost of migration*

In this study we found that farmed silver eels swim at COTs of 34 ± 5 mg $O_2$/kg/km during 2,173 ± 305 km swimming at a swim speed of 0.8 BL/s in FW. The found COTs were very similar to those found for such eels during short term swimming (2 h) in SW (*chapter 2*). With the swim fitness test we found COTs of 38 ± 5 mg $O_2$/kg/km at optimum swim speeds. The same accounted for Lake Grevelingen silver eels swimming at COTs of 52 ± 12 mg $O_2$/kg/km during 1,232 ± 172 km migration and at COTs of 50 ± 8 mg $O_2$/kg/km during the swim fitness test. These results illustrate eel's capability of sustained swim performance. This capacity together with the very high efficiency (4-6 times more efficient than salmon; van Ginneken et al., 2005b and *chapter 2*) make eels ultimate cruising specialists.

Requirements for migration were calculated from body weight loss ($BW_{loss}$) and oxygen consumption ($\dot{M}O_2$) according to formulas in table 1 that are based on energy conversion and oxycaloric values of Brafield & Llewellyn (1982) and bomb calorimetry values of Van Ginneken et al. (2005b). For calculation of energy cost of transport from fat ($COT_{fat}$) from the $BW_{loss}$, we used formulas (6), (7) and (8). For calculation for total energy COT ($COT_{tot}$), we used formulas (9) – (13). For calculation of energy COT from fat ($COT_{fat}$) from the oxygen consumption we used formulas (14) and (15). For calculation for total energy COT ($COT_{tot}$), we used formulas (16) - (20). For extrapolation, we used a standardised distance to the Sargasso of 5,500-km. Farmed eels swim in fresh water (FW) at a total energy cost of transport of 0.469kJ/kg/km (by $\dot{M}O_2$) and 0.522 kJ/kg/km (by $BW_{loss}$; Table 6). The COT of fat is resp. 9.5 and 10.5 mg fat/kg/km. Lake Grevelingen eels swim in salt water (SW) at a higher total cost of transport of 0.702 kJ/kg/km (by $\dot{M}O_2$) and 0.860 kJ/kg/km (by $BW_{loss}$; Table 6). The COT of fat is resp. 14.2 and 17.5 mg fat/kg/km. In literature estimations have been made on costs for eel migration. Table 6 shows an overview of all these experiments. Data were modified on basis of 79.8% fat use (van Ginneken et al., 2005b) as fuel and not as sole energy provider, considered as such in most studies until now. When results are compared, we find that our results concerning simulated migration agree with results of former recent swim experiments (van Ginneken & van den Thillart, 2000; van den Thillart et al., 2004; van Ginneken et al., 2005b), however bomb calorimetry values were found lower (van Ginneken et al., 2005b). Results of these experiments did not agree with the experiments of Schmidt-Nielsen who used however small eels at very high speeds.

*Cost of maturation*

In this study we found that Lake Grevelingen eels incorporate 57 ± 22 g fat/kg (range 33-103 g fat/kg) in the oocytes. For a mean 1-kg silver eels with a fat reserve of 200 g this means that on mean 33% (range 17 to 52%) of the total fat reserve is transported into the gonads. We also calculated these percentages from fat droplet volumes in single oocytes (based on *chapter 5*) with similar results: 25 to 43% of the total fat reserve is incorporated in the oocytes. The values of Boëtius & Boëtius (1980), based on 2 matured females, were low in comparison with this study. Our study widens the range considerably. This is of major importance since the amount of fat transported into the gonads was found to be positively related to the age of the eel. This suggests an increased capacity of older eels to incorporate fat from the muscle into the eggs. This appears to determine the sensitivity to

mature and the start of vittelogenesis since age was negatively correlated to the number of hormonal injections to induce final maturation. As egg quality depends heavily on incorporation of reserves (Adachi et al., 2003), this increased capacity and sensitivity of older eels suggests a greater reproduction potency. As far as we know, age has never been considered as such for fish in literature.

*Cost of reproduction*

The total cost of migration can be calculated by multiplying the energy cost of transport by fat of Lake Grevelingen eels times the distance to the Sargasso of 5,500-km. We can conclude that a 1-kg silver eel requires 60 – 107 g fat for migration. We measured a cost of maturation of 33 – 103 g fat. Sucessful reproduction would require 93 – 210 g fat in total. Since a mean 1-kg silver eel has about 200 g fat (fat percentage of 20%), at least 47% up to 100% of the fat stores are used. Silver eels used in this study had percentages of 11 to 35%. In ecological surveys, similar values were found for the majority of the silver eels (Svedäng & Wickström, 1997) implying that fat requirements are not limiting for reproduction. However, since egg quality depends heavily on incorporation of reserves (Adachi et al., 2003), it might well be that silver eels with lower fat percentages (<15%) will not leave and that a next trial will be performed the year after (Larsson et al., 1990; Svedang & Wickstrom, 1997). According to the results of Schmidt-Nielsen (1972) and Boëtius & Boëtius (1980), only eels with the highest fat percentages would be able to migrate for 5,500-km and mature. The lowest estimate of fat costs would be 185 g fat/kg (Table 6 and 7: 139 g fat/kg for migration and 46 g fat/kg for oocyte incorporation). Spending on average 67% of the fat reserves combined with extensive degeneration of muscles, bone as calcium stores (Yamada et al., 2002) and the digestive tract makes survival after spawning improbable.

In this study we found indications that the body constitution remained constant during maturation. The fat percentage in the muscles remained 20% after complete maturation while major fat incorporation into the gonads occurred. Van Ginneken et al. (2005b) reported that the ratio of body constituents lipid, carbohydrate and protein remained constant during 5,500-km migration. These authors concluded that fat, protein and carbohydrate were metabolised in the same proportion. Considering the fats, this is important while they are required as fuel for continuous swimming, as food reserve for developing embryos, but also to keep neutral buoyancy at great depth. The fat percentage in the muscle remained 20% after treatment, a percentage typical for deep sea fish (Bone et al., 1999).

We can conclude that fat fuel stores of the majority of wild migratory silver eels are sufficient for reproduction; for its 5,500-km migration to the Sargasso sea and maturation reaching GSIs up to 60. However, reproductive success may be higher for the older eels. With this, fat percentage is an important discriminator that deserves more attention. Fat percentage reflects on the trophic habitat quality, generation time and amount of silver eels really migrating to the Sargasso and thus reproducing. An estimate for such numbers is crucial but still lacking.

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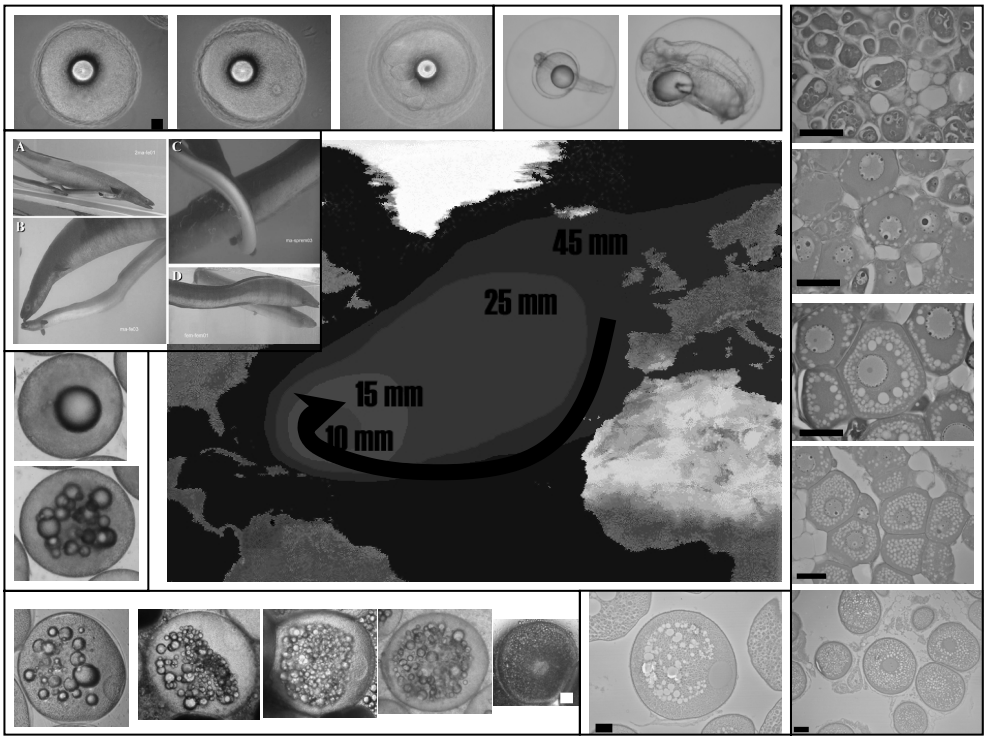


**Figure 6** Synthesis of oocyte development and spawning during and after migration in the Sargasso. During silvering, oocytes start to develop up to the lipid vescicle stage. During freshwater migration, lipids are incorporated during an extended lipid vescicle stage. Vitellogenesis probably occurs alerady near or at the spawning grounds. These are indicated by the decreasing size of the leptocephali found by Schmidt (1923). During the last stages of maturation at the spawning grounds, oocytes hydrate and ovulate. Semelparous spawning occurs collective and simultaneous. Fertilised and dividing eggs rise to the surface. Embryos are supposed to hatch around 60 h.a.f. not including possible delay by high pressure as found by Hiroi et al. (2003).

# Chapter 8
## SUMMARY & RECOMMENDATIONS

**Summary**

In many parts of the world eel is considered a culinary delicacy already since ancient times. Therefore this species is commercially interesting and target for fisheries and aquaculture. Aquaculture of eel is still completely depending on the wild stocks since breeding in captivity is not successful. The wild stocks have however been declining over the past 25 years to a great extent without any signs of recovery. A wide diversity of factors is assumed to be involved determining the quantity (habitat degradation, restocking, overfishing and migratory obstacles) as well as the quality (pollution, introduction of new diseases) of the spawning stocks. A great need for management and conservation measures exist but knowledge on eel biology is incomplete to provide such tools. The lack of knowledge mainly concerns the mysterious oceanic phase in the amazing life cycle of this catadromous fish species.

The European eel *Anguilla anguilla* is one out of 15 species of freshwater eels that all have an oceanic phase characterised by a long distance spawning migration and a semelparous spawn. European eels are born as leptocephalus larvae in the Sargasso Sea. After a journey of seven to nine months they arive at the continental shelf and display a first metamorphosis into glass eels. As immature yellow eels they reside in the estuaries or migrate upstream the European freshwater rivers where they spend a long feeding stage. After reaching a certain age and size, yellow eels cease feeding and start metamorphosis for a second time: the process of silvering turning them into silver eels.

Silvering is a complex phenomenon linking external and internal modifications. Pankhurst (1982) developed an index on the basis of eye size and bodylength to discriminate between yellow and silver eels. Very recently, Durif (et al., 2005) recognized intermediate phases and suggested a silver index based on eye size, pectoral fin length, bodylength and bodyweight. Still a multidisciplinary discussion continues whether silvering is a true metamorphosis, e.g. a marked and abrupt developmental change in the form or structure of an animal, or a more continuous process correlated to the degree of maturation. We observed that the eyes continue to enlarge during artificially induced maturation in a linear fashion (Fig. 1) indicating a more continuous process.

Only after completion of silvering, silver eels leave the continent between September and November and disappear. They migrate probably at depths between 200 and 600 m depth for 5 to 6 months to the spawning grounds in the Sargasso (reviewed by Tesch & Rohlf, 2003), where spawning is believed to occur in March and April (McCleave et al., 1987; McCleave, 2003). Most certainly the effective genitors are characterised by an excellent swim fitness. Highly efficient energy management is required, not only to fullfill migration, but also to provide the eggs with sufficient lipid stores. When the silver eels leave they are still in a prepubertal condition, while after six months swimming they should be fully mature and ready to spawn.

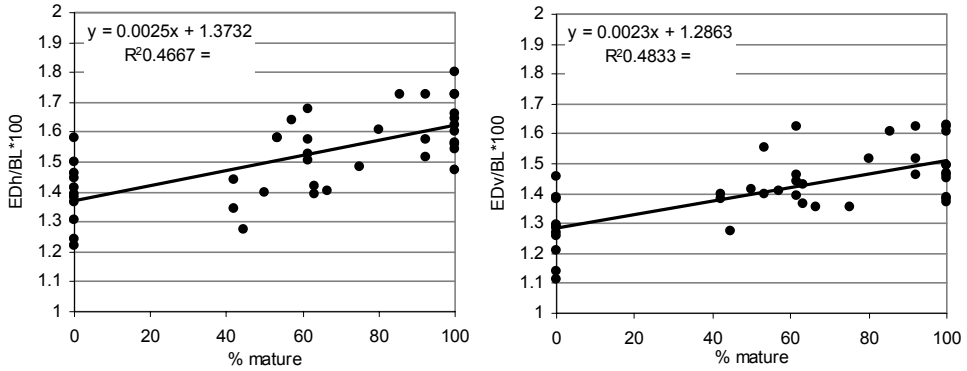


**Figure 1** Eye diameter horizontal (a) and vertical (b) of 11 fully matured females with of each 4 paired observations. The time to reach full maturation is taken as 100%. Both eye diameters horizontal and vertical increase linear and in same ratio.

Information about migration, maturation and the interaction between both is lacking. Understanding of natural triggers for maturation could lead to more successful reproduction protocols.

The general aim of this thesis was to establish the energetic requirements for reproductive migration and maturation of European eel and to assess the role of environmental constraints like the influence of the swim-bladder parasite *A. crassus* on the swim performance and the influence of dioxin-like contaminants on embryonic development.

Biomechanical efficiency of anguilliform swimming is considered low. Experimental studies on the swim efficiency of large migratory silver eels are limited. Very recently, our group found that eels swim 4 to 6 times more efficient than non eel-like fish and utilise *c*. 60g fat per kg for migration (van Ginneken et al., 2005b). Performance is likely to vary among silver eels within and between locations, mainly determined by trophic quality. In order to be able to compare the performance of farmed and wild eels from different locations and under different conditions, we developed a swim fitness test in **chapter 2**. Swim trials with 101 female eels weighing 400 – 1500g were performed in 22 Blazka-type swim-tunnels in a climatised room at 18ºC with running fresh or salt water. Speed and endurance swim trials started at 0.5 up to 1 meter per second (m/s) with increments of 0.1 m/s. Since both tests showed similar results, the single day speed test could be used to predict endurance. Eels showed ability to stabilise and maintain metabolic balance. Although they did not swim fast, they swam highly efficient. Eels reached maximum aerobic swim speeds of 0.81 up to 1.24 BL/s body-length per second (BL/s). At optimum swim speeds of 0.58-0.68 m/s or 0.74-1.02 BL/s cost of transport (COT) values were found of 37-50 mg $O_2$/kg/km which are very low. Energy expenditure during exercise was 20% higher in SW vs FW. Wild silver eels showed lower performance than farmed silver eels. Overall, we can conclude that silver eels can be considered as cruising specialists. If silver eels cruise at optimum swim speeds they would travel for less than 3.5 months to the Sargasso instead of the generally believed 6 months.

Infection with swim-bladder parasite *Anguillicola crassus* is suggested as one of the causes of the collapse of eel populations worldwide. This nematode has been introduced 20 to 30 years ago from Asia and parasitised in short time various eel species in different geographical regions of the world. The effects are energy drain due to its sangivorous activities and mechanical injury of the swim-bladder wall by its migratory activity. These effects are hypothesized to impair spawning migration of European eel. In **chapter 3** we have investigated the effects of infection on swim performance. We hypothesized that parasitic sanguivorous activities reduce swim endurance while the mechanical injury impairs buoyancy control. Eighty eels suffering various degrees of infection have been introduced in the swim-tunnels and subjected to the swim fitness test as developed in chapter 2. Oxygen consumption was measured of large infected silver eels swimming at different speeds allowing to determine swim efficiencies. We found that especially silver eels are targets of infection. Infected eels have lower cruise speeds and higher cost of transport. Eels, that are not infected but contain a swim-bladder damaged by previous infection, show similar effects and even higher cost of transport. Effects thus seem to be associated with swim-bladder disfunction and the resulting loss of neutral buoyancy. This leads to the conclusion that infected eels with damaged swim-bladders have lower success to reach the spawning grounds. Simulated migration trials confirmed fast migration failure (<1,000-km). This study showed that *A. crassus*-infection severely impairs the reproductive potential of eel. Recent studies indicated similar roles for PCB pollution and viruse infection. We can therefore conclude that the downfall of quality (e.g. swim-bladder parasite, EVEX virus, PCBs etc.) of future genitors may well be a major acting force behind the eel's world-wide collapse.

Since European eel *Anguilla anguilla* is one of the most extreme examples of reproductive homing, it is a perfect model to study the poorly understood relation between migration and maturation. In **chapter 4** we investigated this relation. We hypothesized that swimming is involved in metamorphosis (silvering) and release from reproductive inhibition and depressed lipid mobilisation. In this study, we subjected 55 old (>13 years) eels from Lake Balaton (Hungary) to swimming for durations of 1, 2 and 6 weeks. Changes in morphometry and oocyte development were determined to establish the silvering and maturation status. We found that swimming stimulates silvering, shown by enlargement of the eyes already within 2 weeks of swimming. Furthermore, we found that swimming stimulates maturation. Already within 1 week swimming, the gonadal mass increased, oocytes shifted in stage, became larger and large amounts of lipids were incorporated in an extended lipid droplet stage. Synchronisation of oocyte development occurred within and between eels. No indications for vitellogenesis were found. We can conclude that swimming plays a major role in release from reproductive inhibition and mobilisation of lipids to the oocytes. Vitellogenesis and final maturation were not induced and may in the field situation only occur in the surroundings or at the spawning ground itself. Pre-treatment by swimming in protocols to breed eel or other migrant species in captivity may increase sensitivity for hormonal stimulation, fertility and reproductive success.

In **chapter 5**, we artificially matured European eel with the existing protocols for Japanese eel. In Japanese eels, the moment of stimulation of final maturation and ovulation is mainly based on weight increase related to the hydration response of the oocytes, which, in the European eel, is irregular. In contrast to Japanese eel, European eels show wide

individual variability and much slower response to hormonal stimulation. We did not find a difference in response time between Lake Grevelingen and Loire females (Fig. 2) suggesting that this response time is typical for European eels. In this study, the oocyte development of wild European silver eels was followed during final maturation. We describe 7 developmental stages based on 6 parameters: transparency, diameter of the oocyte and position and visibility of the nucleus, and diameter and number of oil droplets. Together, these parameters describe unidirectional changes from immature to over-ripe eggs. The developmental status of the gonads were determined in biopsies from 23 female eels, of which 14 ovulated and were stripped, while 9 gave eggs that could be fertilised. Oocytes matured asynchronously, but this seems to be an artefact, since fertility dropped with every new generation of oocytes. As the timing of ovulation is crucial for fertility of the eggs, our developmental index of oocytes should result in more successful maturation protocols.

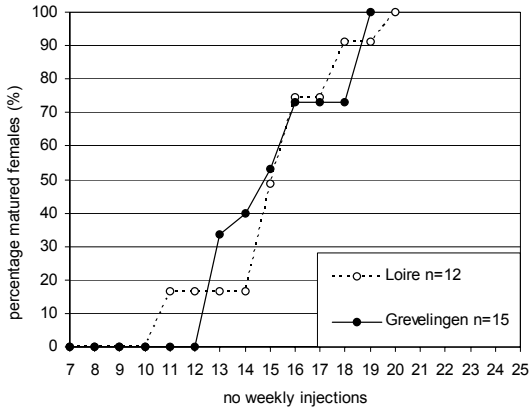


**Figure 2** Rate of maturation. Female silver eels from Lake Grevelingen and River Loire were weekly injected with carp pituitary extract. Maturation was determined from regular egg biopsies. Frequency of occurrence of matured females from Lake Grevelingen vs. Loire River. Similar timing and speed of maturation response exists.

During the first three hours post fertilisation (h.p.f.), most eggs in all batches showed meroblastic cleavage up to the eight cell stage. Egg batches of two females resulted in the development of about 1600 embryos at 31-32 h.p.f. (Fig. 3). Embryos of one female (n=100) continued to develop and were found vigorously moving with the pigmented tail at 58-60 h.p.f indicating the onset of hatching. At this time they showed a yolk sac in which the protein part had disappeared and only the fat droplet remained (Fig. 4). Embryonic development continued until 100 h.p.f. when last embryos died. Hatching was not observed.

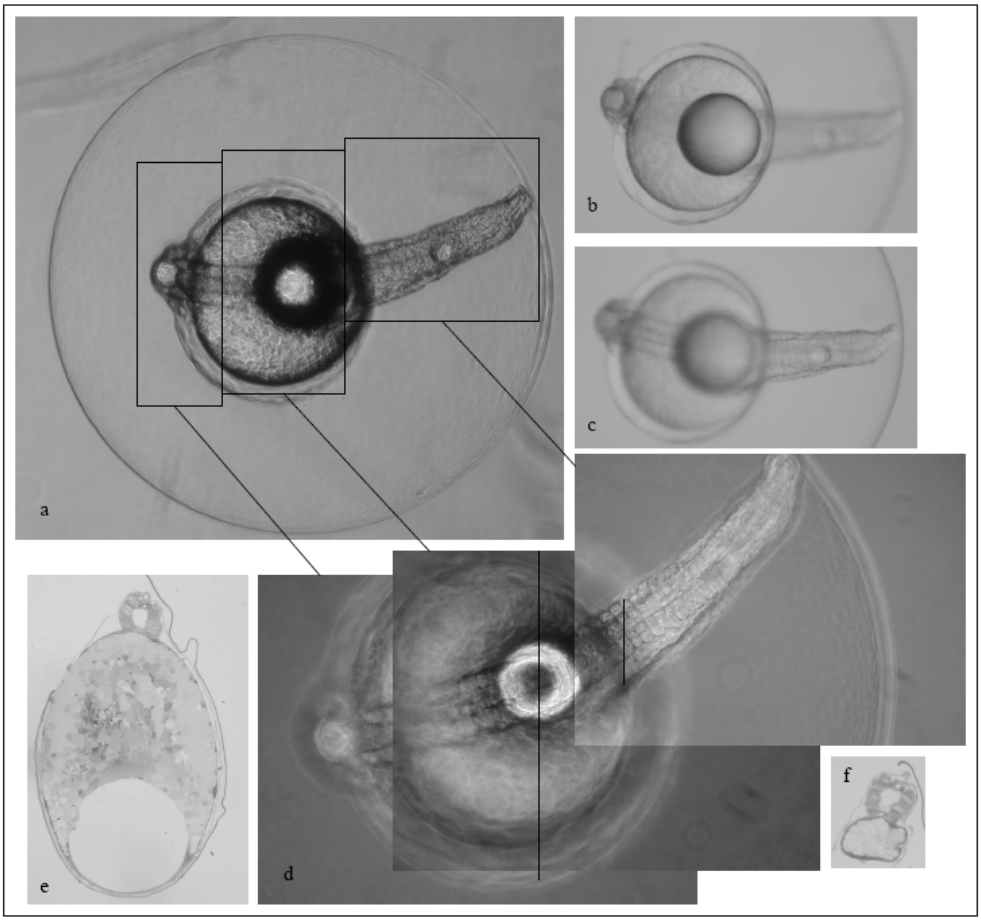


**Figure 3** Stretched embryo at 32 h.p.f. photographed with a) phase contrast microscopy and b) and c) with bright field microscopy, d) zoomed-in views showing the developing somites and in e) and f) cross-sections at two locations as illustrated.

Embryos of a second successful female (n=1500) showed serious oedema of the yolksac, a deformed head region and absence of a heartbeat. Such embryonic malformations are typical for PCB-exposed eggs and indicate negative inteference with dioxin-like contaminants. Therefore in **chapter 6** we measured parental levels of dioxin-like contaminants and correlated their distribution to embryonic survival and development. The total dioxin-like toxic potency of the individual gonad batches was determined as TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxine) – equivalents (TEQs), using an in vitro reporter gene assay. The observed differences in development and survival showed a significant negative correlation with the TEQ levels in the gonads, already at levels far below the maximal allowable level for fish consumption i.e. 4 ng TEQ/kg fish. The clear inverse relationship between the TEQ-level and the survival period of the fertilised eggs

strongly suggest that the current levels of dioxin-like compounds seriously impair the reproduction of the European eel. The peak of the environmental levels of dioxin-like PCBs and the decline of eel coincide world-wide, further suggest that, in addition to other threats, these contaminants contributed significantly to the current collapse of eel populations.

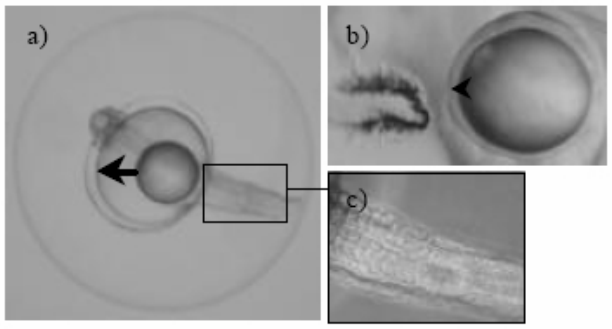


**Figure 4** Eel embryos. a) arrows indicate embryonic yolk protein in an embryo at 32 h.p.f. b) yolk protein was absent in an embryo at 60 h.p.f. showing also the pigmented tail. c) detailed view of developed somites and Kupfer organ (phase contrast microscopy).

Few attempts were made to estimate the energetic costs of migration and maturation of European eel. The stored energy of silver eels, mainly as lipids in muscle and under the skin (Fig. 5), should suffice for successful reproduction. In **chapter 7**, we therefore subjected cultured eels and wild large silver eels to simulated migration at different speeds and calculated the cost of transport (COT) from oxygen consumption rates. We found that cultured eels swam at COTs of 34 ± 5 mg $O_2$/kg/km during 2,173 ± 305 km migration. Wild silver eels swam at higher COTs of 52 ± 12 mg $O_2$/kg/km during 1,232 ± 172 km migration. COTs were rather constant and similar to values of short term 2h swim tests. Wild silver eels spend 78 ± 4 g fat /kg, or 39% of the fat stores at average fat percentages of 20%, on complete 5,500-km migration. These relatively low values confirm their high swim efficiency. Furthermore, we artificially matured eels from the same batch of wild silver eels by hormonal injections to determine the amount of fat incorporated in the oocytes. We found that eels incorporate 57 ± 22 g fat /kg, or 28% of the fat stores, in the oocytes which is positively related to age. Thus in total 67% of the fat stores is spent on the eel's spawning run (Fig. 6). Fat requirements of average silver eels from high quality trophic habitats are thus not limiting for reproduction.

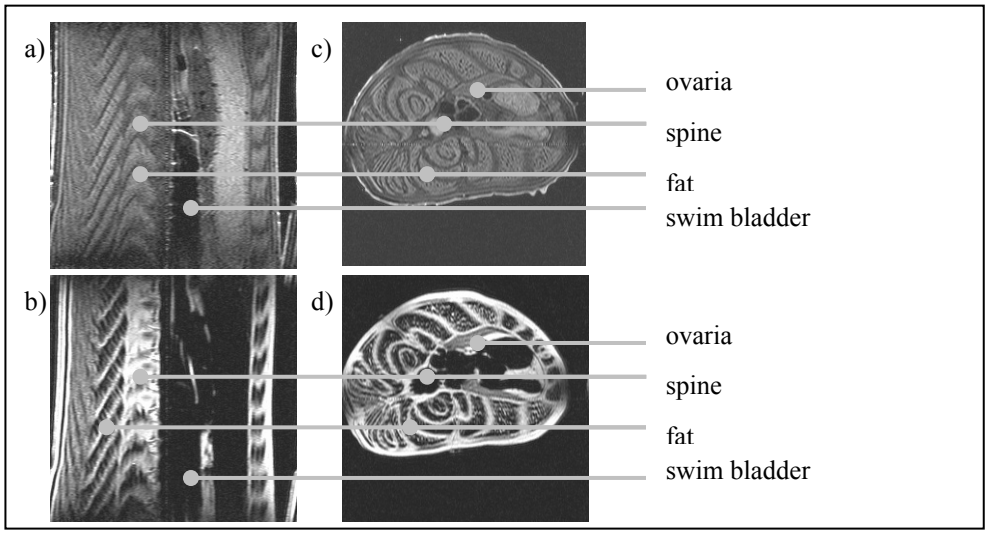


**Figure 5** MRI with a 5.6 Tesla magnet (300 MHz) of eel showing where fat is stored with a) water image longitudinal, b) fat image longitudinal, c) water image cross-sectional and d) fat image cross-sectional. (Performed in collaboration with Prof. Dr. Annemie van der Linden, University of Antwerp, Belgium).

From this we can conclude that:

1) Silver eels do not swim fast but highly efficient at all speeds as we found with a developed swim fitness test. Cost of transport is very low and remains constant during migration. Silver eels are therefore cruising specialists. Especially silver eels are target of *A. crassus* infection and show lower cruise speeds and higher cost of transport. The nematodes cause damage on the swim-bladder wall that results in loss of neutral buoyancy and likely migration failure. Swimming releases eel from reproductive inhibition and stimulates mobilisation of lipids to the oocytes.
2) Timing of ovulation may be improved and fertility increased by applying the developed oocyte developmental index. We succeeded in fertilising batches of eggs multiple times and raised embryos up to 4 days after fertilisation. Differences in embryonic development and survival showed negative correlation with dioxin-like contaminant levels in the gonads already at low levels.
3) Fats are mobilised for 39% as fuel and are for 28% incorporated in the oocytes at the same time. Age is a determimant for successful reproduction since a) older eels showed increased capacity to incorporate more fat from the muscle into the oocytes determining higher egg quality, b) older eels are more sensitive for hormonal stimulation, and c) older eels are more susceptible to swimming induced oocyte development.

CHAPTER 8

**Recommendations for the protection and restoration of eel stocks**

Silvering prepares eels for their oceanic migration indicated by the improved tolerance for seawater transfer and to pressure (Fig. 7). However, no indications were found in this study for improved swim performance of silver eels as compared to yellow eels (chapter 2). Swim exercise however induced silvering and maturation (chapter 4; Fig. 7). Only silver eels were able to complete maturation (Fig. 7). The oldest eels with the highest body weight and condition factor (highest fat percentage) showed highest sensitivity for hormonal stimulation (chapter 6 and 7).

Two strategies may be followed for the protection and restoration of eel stocks: 1) to protect the natural populations especially the silver eels contributing to reproduction, and 2) to stimulate research on artificial reproduction of eels in captivity. Concerning point 1, the quality of the habitat determines the quality of the targeted silver eels (the oldest and fattest). Silver eels should be free from swim-bladder parasite *A. crassus* (this study chapter 3) and viruses like EVEX (van Ginneken et al., 2004, 2005c; Fig. 7) since they impair migration. Furthermore, they should be free from dioxin-like contaminants since these negatively interfere with migratory capacity and have devastating effects on survival and development of offspring (this study chapter 6; Fig. 7). Recommendations therefore involve:

1. Monitoring of the production of the targeted silver eels in each EU-memberstate to estimate the reproductive potential of European eels.
2. Hydrosystems that produce high proportions of the targeted silver eels should be protected.
3. Dioxin-like contamination should be monitored in targeted silver eels of all major hydrosystems and targeted silver eels from areas with low levels should be protected. Actions should be taken to reduce levels.
4. Targeted silver eels from all major hydrosystems should be monitored for viruses and swim-bladder parasites and targeted silver eels from virus free areas with low parasite loads should be protected. Only virus free eel transports should be allowed.
5. Targeted silver eels should be provided migratory passage free from fisheries and obstacles.

**Tools for the future of successful artificial reproduction**

Success rates in artificial reproduction are still low for the European eel and not only because of disturbing effects of contaminants. The same accounts for Japanese eels although larvae can be bred. Adachi et al. (2003) stated that '*artificially matured Japanese eels exhibit many peculiarities such as variations in yolk accumulation and egg membrane formation, differences in the process of oocyte maturation and serum hormone levels, and other phenomena. These variations seem to indicate abnormality rather than species specificity*'. In artificially matured Japanese eels, vitellogenesis occurs when oocytes measure $\geq$ 250 μm, yolk globuli accumulate and the chorion thickens. The number of fat droplets still increases during vitellogenesis, which is considered not normal (Adachi et al., 2003). New Zealand longfinned eels are more matured when they leave for their spawning grounds. Their oocytes have more oil droplets, less yolk globuli and a thinner chorion in comparison to oocytes of artificially matured Japanese eels. Adachi et al. (2003) concluded that artificially matured oocytes of *A. japonica* enter vitellogenesis at an earlier stage.

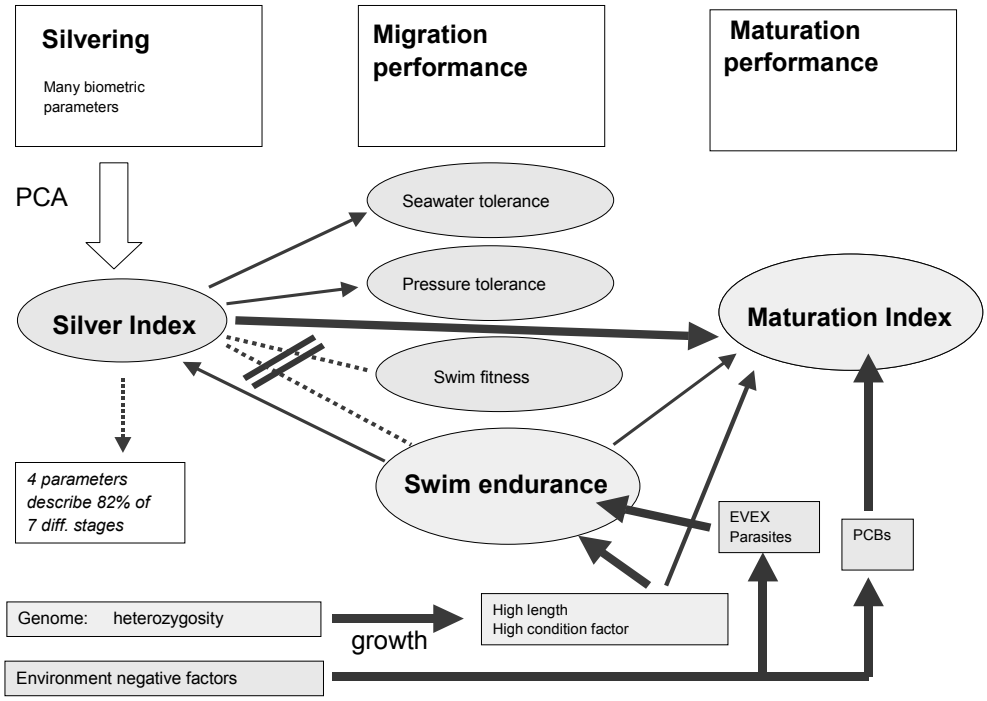


**Figure 7** Inter-relationships between the silvering and migration/maturation performance. High silver index correlates with an improved sea water and high pressure tolerance, however, no effect was evident on the swim fitness. High silver index correlates with high maturation index. Large size and high fat content improves maturation as well as swim endurance. Furthermore, it was found that swimming induced silvering and maturation. Negative environmental factors affect the swim endurance and the maturation index. Infections with EVEX/parasites are devastating for swimming eels, while PCBs impair fertility. Taken from the summary and recommendations of the EELREP project.

**Table 1** Identification tabel for the reproduction capacity of female European silver eels. Fat content as % of wet weight; Silver stages of female eels are based on silver index scores. Reproduction capacity is indicated from very likely (******) to absent (0). Taken from the summary and recommendations of the EELREP project.

| Silver stage | I | II | III | IV | Va | Vb |
|---|---|---|---|---|---|---|
| Body length | -- | -- | -- | -- | <70cm | >70cm |
| Fat content | -- | -- | -- | < 13% | 13- 20% | >20% |
| Reproductive capacity | 0 | 0 | 0 | * | *** | ****** |

Providing the offspring with the necessary reserves of fats and proteins is crucial for successful reproduction. The observed differences indicate abnormality of the maturation process.
Thus although the protocol for breeding of eels in captivity is more successful for Japanese eel at this moment, still numerous problems are encountered that can be traced back to the maturation process of the parents. In order to come to a successful breeding protocol, there is a need for knowledge on natural oocyte development, its triggers and its regulation. This study has provided substantial new insight.

I. Selection of eels

This study provided criteria for selection of eels that can be used; Eels should be free from (dioxin-like) contamination. Only European eels from Sardinia and some locations in Ireland fit these criteria nowadays. Trophic habitat quality needs to be high, so silver eels are able to store enough fat reserves. Selected eels should be relatively old (>10 years) since we found increased capacity to incorporate more fat from the muscle into the eggs determining higher egg quality.
Additionaly, in EELREP was showed that correlations existed between body length and silver stage (Durif et al., 2005) with the hormone sensitivity. Large (>70 cm) migratory females (stage $S_{FV}$) with fat percentages >20% were considered most suitable (Table 1).

II. Increasing maturation sensitivity

This study showed that sensitivity was increased by swimming. Already short term swimming caused 1) release from reproductive inhibition, and 2) mobilisation of fat to the oocytes. Swimming induced incorporation of fats in the oocytes, without inducing vitellogenesis, apparently requires a long swim period. CPE injection induces vitellogenesis almost immediately in oocytes (in European eel after two injections; Palstra et al., unpublished results) that might not be in an appropriate state yet e.g. fat incorporation needs to be finalised. The CPE used in these experiments comes from spawnable carps and other hormones besides LH and FSH may have early effects. Pre-treatment by swimming in protocols to breed eel or other migrant species like salmon (Patterson et al., 2004) in captivity may increase sensitivity for hormonal stimulation, fertility and reproductive success.

III. Improve timing

With the existing Ohta (et al., 1996) protocol, ovulation does not occur spontaneously but needs to be induced. This experimenter needs to make the decision when the time is right and not the eels themselves. In Japanese eels, the moment of stimulation of final maturation and ovulation is mainly based on weight increase related to the hydration response of the oocytes, which, in the European eel, is irregular. The oocyte development index that we developed can be applied instead to improve timing of ovulation and increase fertility. Antoher approach is not to strip but to let eels themselves determine timing of spawning through the action of pheromones by joining matured males and females.

IV. New techniques

One important disadvantage of weekly hormonal induction is the large amount of handling stress. It is well known that stress reduces fertility, so low stress must improve succes. Recent innovations make it possible to circumvent this disadvantage. Leiden University recently patented hormone (LH/FSH) producing cells which can be implanted. Other approaches involve GnRH implants that stimulate the pituitary to produce LH and FSH or gene transfer.

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# Nederlandse Samenvatting

## Energievereisten en omgevingsbeperkingen van reproductieve migratie en maturatie van de Europese schieraal (*Anguilla anguilla* L.)

In menig deel van de wereld wordt de aal als sinds de oudheid beschouwd als culinaire delicatesse. Daardoor dient de aal een commercieel belang en fungeert het als een doel voor visserij en aquacultuur. Aal aquacultuur is nog altijd volledig afhankelijk van het wilde bestand omdat het kweken in gevangenschap nog niet succesvol is. De wilde populatie vertoont echter een sterke afname over de laatste 25 jaar zonder enig teken van herstel. Een brede diversiteit aan factoren wordt verondersteld betrokken te zijn die ofwel de kwantiteit (habitatreductie, heruitzetting, overbevissing en migratie barrières) dan wel de kwaliteit (vervuiling, introductie van ziekten) bepalen van de jaarlijkse hoeveelheid paaiers. Een grote behoefte bestaat aan het nemen van management en beschermingsmaatregelen maar de kennis van de biologie van de aal vertoont hiervoor nog te grote hiaten. Het gebrek aan kennis betreft vooral de mysterieuze oceanische fase in de intrigerende levenscyclus van deze katadrome vissoort.

De Europese aal *Anguilla anguilla* is één van de 15 soorten 'zoetwater' alen die allen een oceanische fase gemeen hebben, gekenmerkt door een lange afstandsmigratie en een eenmalige semelpare paai. Europese alen worden geboren als Leptocephalus larven in de Sargasso Zee. Na een reis van 7 tot 9 maanden arriveren ze op het Europees continentaal plat en vertonen een eerste metamorfose naar glasaaltjes. Als onvolwassen rode alen verblijven ze in de estuaria of migreren stroomopwaarts in de Europese zoete binnenwateren waar ze een lang voedingsstadium kennen. Na het bereiken van een bepaalde leeftijd en grootte stoppen de rode alen met eten en ondergaan een tweede metamorfose: het schierwordings of 'silvering' proces dat ze doet veranderen in schieraal.

Het schier worden is een complex fenomeen dat bestaat uit externe en interne modificaties. Pankhurst (1982) ontwikkelde een index op basis van ooggrootte en lichaamslengte om onderscheid te maken tussen rode en schiere alen. Recentelijk heeft Durif (et al., 2005) additioneel onderscheid gemaakt in intermediaire fasen en heeft een 'silver' index voorgesteld op basis van ooggrootte, lengte van de pectorale (borst)vinnen, lichaamslengte en lichaamsgewicht. Nog altijd voltrekt zich een multidisciplinaire wetenschappelijke discussie of schier worden nu een echte metamorfose betreft, ofwel een duidelijke en abrupte verandering in ontwikkeling van vorm of structuur van een dier, of een meer continu proces dat gecorreleerd is aan de mate van maturatie of rijping. We observeerden dat de ogen tijdens kunstmatig geïnduceerde maturatie groter bleven worden op een lineaire manier wat een meer continu proces indiceert.

Pas na het completeren van het proces verlaten de schiere alen het continent vooral tussen september en november en verdwijnen in de oceaan. Ze migreren waarschijnlijk op diepten variërend tussen de 200 en 600 meter gedurende 5 tot 6 maanden naar de paaigronden in de Sargasso (overzicht door Tesch & Rohlf, 2003), waar de paai plaats heeft in maart en april (McCleave et al., 1987; McCleave, 2003). Hoogstwaarschijnlijk worden

de effectief reproducerende alen gekenmerkt door een excellente zwem fitness. Een zeer efficiënte energie huishouding is vereist, niet alleen om te voldoen aan de migratie, maar ook om de eieren te voorzien van voldoende vetvoorraden. Als de schiere alen vertrekken zijn ze nog altijd slechts in prepuberale staat terwijl ze na 6 maanden zwemmen volledig gematureerd en afgerijpt dienen te zijn en klaar zijn om te reproduceren.

Informatie over migratie, maturatie en de interactie tussen beiden ontbreekt. Het begrijpen van de natuurlijke impulsen tot maturatie zou kunnen leiden tot meer succesvolle reproductieprotocollen. Het voornaamste doel van dit proefschrift is om de energievereisten voor reproductieve migratie en maturatie vast te stellen en om de rol te bepalen van omgevingsfactoren zoals de invloed van de zwemblaasparasiet *Anguillicola crassus* op de zwemprestatie en de invloed van dioxineachtige contaminanten op embryonale ontwikkeling.

Biomechanische efficiëntie van anguilliform zwemmen wordt als laag beschouwd. Experimentele studies van de zwemefficiëntie van grote migratoire schieralen zijn zeer beperkt. Zeer recentelijk vond onze groep dat alen 4 tot 6 keer zo efficiënt zwemmen als niet-aalachtige vissen en ongeveer 60 gram vet per kilogram aal gebruiken voor migratie (van Ginneken et al., 2005b). Prestaties worden verondersteld te variëren tussen individuele schieralen, binnen en tussen locaties, voornamelijk bepaald door de trofische kwaliteit van het habitat. Om de zwemprestatie te vergelijken tussen kweekalen en wilde alen van diverse locaties en onder verschillende condities hebben we in **hoofdstuk 2** een zwemfitness test ontwikkeld. Zwemtesten met 101 vrouwelijke alen met een gewicht van 400 – 1500 gram werden uitgevoerd in 22 zwemtunnels van het Blazka-type, geplaatst in een geklimatiseerde cel, in zoet of zout water bij een temperatuur van 18ºC. Zwemsnelheid en uithoudingsvermogen werden getest bij snelheden van 0.5 tot 1 meter per seconde (m/s) met stappen van 0.1 m/s verhoging. Omdat beide testen soortgelijke resultaten hadden kon de zwemsnelheidstest, die slechts een dag in beslag neemt, worden gebruikt om uithoudingsvermogen te voorspellen. Alen vertoonden het vermogen om de metabolische balans te stabiliseren en te handhaven. Hoewel ze niet snel zwommen, zwommen ze erg efficiënt. Alen bereikten maximale aerobe zwemsnelheden van 0.81 tot 1.24 lichaamslengten per seconde (body-lengths per second, BL/s). Bij optimale zwemsnelheden van 0.58-0.68 m/s, of 0.74-1.02 BL/s, werden erg lage transportkosten (cost of transport, COT) gevonden van 37-50 mg $O_2$/kg/km. Energiekosten tijdens zwemmen waren 20% hoger in zout water dan in zoet water. Wilde alen presteerden slechter dan kweekalen. Over het algemeen kunnen we concluderen dat schieralen kunnen worden beschouwd als steeërs of ‘cruisers’; specialisten op de lange afstand. Als vrouwelijke schieralen continu zouden ‘cruisen’ bij optimale zwemsnelheden, dan zouden ze minder dan 3.5 maanden onderweg zijn naar de Sargasso in plaats van de algemeen aangenomen 6 maanden zwemmen.

Er is gesuggereerd dat infectie met de zwemblaasparasiet *Anguillicola crassus* een van de oorzaken zou zijn van het wereldwijde instorten van de aal populaties. Deze nematode werd 20 tot 30 jaar geleden geïntroduceerd vanuit Azië en parasiteerde binnen korte tijd diverse aalsoorten in verschillende geografische gebieden van de wereld. Effecten zijn energieverlies vanwege de sanguivore activiteiten van de parasiet en mechanische schade van de zwemblaaswand door de migratoire activiteiten van de parasiet. Deze effecten worden verondersteld de paaimigratie van de Europese aal te beletten. In **hoofdstuk 3** hebben we de effecten van de infectie op de zwemprestatie onderzocht. We hypotheseerden dat de parasitaire sanguivore activiteiten het uithoudingsvermogen zouden

reduceren terwijl de mechanische schade het drijfvermogen zou inperken. Tachtig alen die leidden aan variërende maten van infectie werden geïntroduceerd in de zwemtunnels en onderworpen aan de zwemfitnesstest zoals beschreven in hoofdstuk 2. De zuurstofconsumptie werd gemeten van deze grote geïnfecteerde schieralen bij diverse zwemsnelheden en stelde ons in staat de zwemefficiënties te bepalen. We vonden dat vooral schieralen doel van infectie zijn. Geïnfecteerde alen hadden lagere 'cruise' snelheden en hogere transportkosten. Alen die niet waren geïnfecteerd maar wel zwemblaasschade vertoonden door voormalige infectie, vertoonden gelijke effecten en zelfs hogere transportkosten. Effecten lijken dus vooral geassocieerd te zijn met het niet functioneren van de zwemblaas en het resulterende verlies van drijfvermogen. Dit leidt tot de conclusie dat geïnfecteerde alen met beschadigde zwemblazen een verminderd succes hebben om de paaigronden te bereiken. Gesimuleerde migratie testen hebben een snelle migratiemislukking bevestigd (<1,000-km). Deze studie heeft laten zien dat *A. crassus*-infectie het reproductieve potentieel van de aal zeer nadelig beïnvloed. Recente studies hebben een soortgelijke rol voor PCB verontreiniging (van Ginneken et al., submitted) en virus infectie (van Ginneken et al., 2004, 2005a) geïndiceerd. Daarom kunnen we concluderen dat het verlies van kwaliteit (zwemblaasparasiet, EVEX virus, PCBs ed) van toekomstige paaiers wel eens de belangrijkste factor achter het instorten van de wereldwijde aalpopulaties zou kunnen betekenen.

Omdat de Europese aal een van de meest extreme voorbeelden is van reproductieve 'homing', het terugkeren naar de geboortegronden om daar te reproduceren, is het een perfect model om de nauwelijks begrepen relatie tussen migratie en maturatie te onderzoeken. In **hoofdstuk 4** hebben we deze relatie onderzocht. We hypotheseerden dat zwemmen betrokken is in metamorfose (schierwording) en ontheffing van reproductieve onderdrukking en onderdrukte vetmobilisatie. In deze studie hebben we 55 oude (>13 jaar) alen van het Balaton meer (Hongarije) laten zwemmen voor een duur van 1, 2 en 6 weken. Veranderingen in morfometrie en eicelontwikkeling werden bepaald om de schierheids- en maturatie status vast te stellen. We vonden dat zwemmen schierwording stimuleert, aangegeven door het vergroten van de ogen reeds binnen 2 weken zwemmen. Bovendien vonden we dat zwemmen de maturatie stimuleert. Al binnen een week zwemmen was de gonadenmassa toegenomen, eicellen veranderden naar een verder ontwikkelingsstadium, ze werden groter en grote hoeveelheden vet werden geïncorporeerd tijdens een uitgebreide 'lipid droplet stage'; het eicelontwikkelingsstadium gedurende welke vetinbouw plaats heeft. Synchronisatie van eicelontwikkeling had plaats in en tussen alen. Er werden geen aanwijzingen gevonden voor 'vitellogenese'; het eicelontwikkelingsstadium gedurende welke proteïne-inbouw plaats heeft en welke volgt op de 'lipid droplet stage'. We kunnen concluderen dat zwemmen een belangrijke rol speelt in ontheffing van reproductieve onderdrukking en mobilisatie van vet naar de eicellen. Vitellogenese en finale maturatie waren niet geïnduceerd en zouden in het veld alleen kunnen plaats hebben in de buurt of op de paaigronden zelf. Voorbehandeling door zwemmen in protocollen die ervoor dienen de aal of andere migratoire soorten in gevangenschap te kweken zouden de sensitiviteit voor hormonale behandeling, de fertiliteit en het reproductieve succes kunnen doen toenemen.

In **hoofdstuk 5** hebben we de Europese aal artificieel gematureerd met de bestaande protocollen voor de Japanse aal. In de Japanse aal is het moment van stimulatie van finale maturatie en ovulatie voornamelijk gebaseerd op een gewichtstoename die is gerelateerd aan de hydratatierespons van de eicellen die, in de Europese aal, onregelmatig

is. In tegenstelling tot de Japanse aal laat de Europese aal een veel wijdere individuele variabiliteit zien en een veel langzamere respons op hormonale stimulatie. We vonden geen verschil in responstijd tussen vrouwtjes van het Grevelingen meer en de Loire wat suggereert dat deze respons typisch is voor de Europese aal. In deze studie is de eicelontwikkeling bij wilde Europese schieralen gevolgd tijdens de finale maturatie. We beschrijven 7 ontwikkelingsstadia die gebaseerd zijn op 6 parameters: transparantie, eiceldiameter, positie en zichtbaarheid van de kern, en diameter en aantal vet druppels. Gezamenlijk geven deze parameters een unidirectionele verandering weer van onvolwassen tot overrijpe eieren. Het ontwikkelingstadium van de gonaden werd bepaald in biopsies van 23 vrouwelijke alen, waarvan er 14 ovuleerden en werden afgestreken en waarvan 9 alen eieren gaven die bevrucht konden worden. Eicellen matureerden asynchroon, maar dit lijkt een artefact aangezien de fertiliteit kelderde met elke nieuwe generatie eicellen. Omdat de timing van ovulatie cruciaal is voor de fertiliteit van de eieren, zou onze eicelontwikkelingsindex kunnen resulteren in succesvollere maturatie protocollen.

Tijdens de eerste 3 uur na bevruchting (hours post fertilisation h.p.f.) lieten de meeste eieren in alle 'batches' (legsels) meroblastische klieving zien tot het 8 cellen stadium. Eibatches van 2 vrouwtjes resulteerden in de ontwikkeling van ongeveer 1600 embryo's na 31-32 h.p.f. Embryo's van één vrouwtje (n=100) bleven ontwikkelen en bewogen energiek met de gepigmenteerde staart 58-60 h.p.f. ter indicatie van het moment van uitkomen. Op dit moment vertoonden ze een dooierzak waarin het proteïnedeel was verdwenen en alleen de vetdruppel bleef over. Embryonale ontwikkeling continueerde tot 100 h.p.f. toen de laatste embryo's stierven. Uitkomen is niet waargenomen.

Embryo's van een tweede succesvol vrouwtje (n=1500) lieten ernstige oedeemvorming zien van de dooierzak, een gedeformeerde kopregio en afwezigheid van de hartslag. Zulke embryonale afwijkingen zijn typisch voor PCB-blootgestelde eieren en indiceren een negatieve interferentie met dioxineachtige contaminanten. Daarom meetten we in **hoofdstuk 6** de niveaus van dioxineachtige contaminanten in de ouders en correleerden deze aan overleving en ontwikkeling van embryo's. De totale dioxineachtige toxische potentie van de individuele gonaden batches werd bepaald als TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxine) – equivalenten (TEQs) met behulp van een in vitro reporter gen assay. De waargenomen verschillen in ontwikkeling en overleving lieten een significant negatieve correlatie zien met TEQ niveaus in de gonaden, zelfs bij niveaus die ver onder het maximaal toelaatbare niveau voor visconsumptie liggen van 4 ng TEQ/kg vis. De duidelijk inverse relatie tussen TEQ niveau en overlevingsperiode van de bevruchte eieren suggereert sterk dat de huidige niveaus van dioxineachtige stoffen de reproductie van de Europese aal ernstig bedreigen. De piek van dioxineachtige PCB's in het milieu en de afname van aal vallen wereldwijd samen dat verder suggereert dat, naast andere bedreigingen, deze contaminanten significant hebben bijgedragen aan het huidige instorten van de aalpopulaties.

Enkele pogingen werden gedaan om de energiekosten voor migratie en maturatie van de Europese aal te schatten. Opgeslagen energievoorraden van schiere alen, vooral als vet in de spieren en onder de huid zouden moeten voldoen voor succesvolle reproductie. In **hoofdstuk 7** hebben we daarom grote schiere kweekalen en wilde alen onderworpen aan gesimuleerde migratie bij verschillende snelheden en we hebben de transportkosten (cost of transport COT) berekend uit zuurstofconsumptiewaarden. We vonden dat kweekalen zwommen bij COT waarden van 34 ± 5 mg $O_2$/kg/km gedurende 2,173 ± 305 km migratie.

Wilde schieralen zwommen bij hogere COT waarden van 52 ± 12 mg $O_2$/kg/km gedurende 1,232 ± 172 km migratie. COT waarden waren vrij constant en gelijk aan waarden die tijdens korte 2 uur zwemtesten werden gevonden. Wilde schieralen spenderen 78 ± 4 g fat /kg, of 39% van de vetvoorraden bij gemiddelde vetpercentages van 20%, aan complete 5500 km migratie. Deze relatieve lage waarden bevestigen de hoge zwemefficiëntie. Bovendien hebben we wilde schieralen van dezelfde lichting kunstmatig tot maturatie gebracht door hormonale injecties om zo de hoeveelheid vet te bepalen die werd ingebouwd in de eicellen. We vonden dat alen 57 ± 22 g fat /kg, of 28% van de vetvoorraden, in de eicellen inbouwen wat positief is gerelateerd aan leeftijd. Dus totaal wordt 67% van de vetvoorraden gespendeerd aan aalpaai. Vetvereisten van gemiddelde schieralen van habitats met hoge trofische kwaliteit limiteren dus niet de reproductie.

Op basis hiervan kunnen we concluderen dat:

1) Schieralen zwemmen niet snel maar hoogst efficiënt bij alle snelheden zoals we vonden met een ontwikkelde zwemfitness test. Transportkosten zijn erg laag en blijven constant gedurende gesimuleerde migratie. Schieralen zijn daardoor lange afstandsspecialisten of 'cruisers'. Vooral schieralen zijn het doel van *A. crassus* infectie en geïnfecteerde alen hebben een lagere cruise snelheid en hogere transportkosten. De parasieten veroorzaken schade aan de zwemblaaswand die resulteert in het verlies van drijfvermogen en waarschijnlijk migratiemislukking. Zwemmen ontheft de aal van onderdrukte reproductie en stimuleert mobilisatie van vet naar de eicellen.
2) Vetten worden gemobiliseerd voor 39% als brandstof en voor 28% ingebouwd in de eicellen. Leeftijd is een determinant voor succesvolle reproductie aangezien oudere alen a) gevoeliger zijn voor zwemmen geïnduceerde eicelontwikkeling, b) een toegenomen capaciteit vertonen om vet van de spieren in de eicellen in te bouwen wat een hogere eicel kwaliteit bepaalt en c) gevoeliger zijn voor hormonale stimulatie.
3) De timing van ovulatie kan worden verbeterd en de fertiliteit worden verhoogd door de ontwikkelde eicelontwikkelingsindex toe te passen. We zijn in staat geweest vele keren eibatches te bevruchten en hebben embryo's gekweekt tot 4 dagen na bevruchting. Verschillen in embryonale ontwikkeling en overleving vertoonden een negatieve correlatie met niveaus van dioxineachtige contaminanten, reeds bij lage niveaus.

In het afsluitende **hoofdstuk 8** worden, volgend op de samenvatting, aanbevelingen ter bescherming en restauratie van aalpopulaties gesuggereerd op basis van het onderzoek zoals gepresenteerd in dit proefschrift en in breder EU verband. Deze betreffen met name waarborging van de kwaliteit van de schieraal: 1) Het monitoren van de productie aan hoog kwalitatieve migratoire schieraal in elke EU lidstaat met als doel het reproductieve potentieel van de Europese aal vast te stellen, 2) Het beschermen van hydrosystemen die aanzienlijke proporties van deze hoog kwalitatieve migratoire schieralen produceren, 3) Het monitoren van dioxineachtige contaminatie in migratoire schieralen van alle belangrijke hydrosystemen en het beschermen van migratoire schieralen van gebieden met lage niveaus. Actie moet worden ondernomen om maatregelen ter reductie van deze niveaus te ondersteunen, 4) Het monitoren van virussen en zwemblaasparasieten in migratoire schieralen van alle belangrijke hydrosystemen en het beschermen van migratoire schieralen met lage parasiet niveaus van virus vrije gebieden, 5) Het verlenen van doorgang aan migratoire schieralen, vrij van visserij en barrières.

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**Spakenburg Paling BV**

# Curriculum Vitae

Arjan Peter Palstra werd geboren in Den Haag op 28 februari 1973 onder het sterrenbeeld vissen terwijl zijn zussen Stuif-es-in zaten te kijken. Het eerste dat hij deed op deze aardkloot was een plasje plegen waardoor iedereen al snel wist dat het een jongen was. Zijn fascinatie voor het onderwaterleven werd gesterkt door het zomerhuisje aan de Reeuwijkse plassen waar de weekenden en vakanties werden doorgebracht. Het sportvissen werd sterk gestimuleerd door de door zijn moeder verstrekte subsidies van 5 cent per vis. Al snel werd specialistisch gejaagd op karper en snoek terwijl de paling altijd werd verwelkomd voor de pan. In het eindexamenjaar overleed zijn vader Peter Palstra op 53 jarige leeftijd. Dat jaar werden certificaten gehaald voor 4 vakken, waaronder biologie, aan het St. Janscollege te Den Haag. Steun van zijn school in dit moeilijke jaar heeft hij niet gehad. Het jaar daarop werden nog eens 4 certificaten gehaald aan het Noctua in Scheveningen. In 1993 begon hij met de studie Biologie aan de Universiteit Utrecht. Even maakte hij een schijnbeweging naar Algemene Letteren maar daarna vervolgde hij de studie Biologie met een moleculair-zoölogisch profiel. In 1997 nam hij een eerste belangrijke beslissing door te besluiten de studie Biologie te combineren met zijn hobby vissen. Vervolgens begon hij zijn eerste 9-maands stage bij de vakgroep Visteelt aan de Wageningen Universiteit. Hij bestudeerde de "Genetical characterisation of androgenetic carp (*Cyprinus carpio* L.)" onder supervisie van Dr. Hans Komen and Dr. Michael Tanck. Hij ontving hiervoor een 9 en later werd deze studie gepubliceerd in het wetenschappelijk vakblad Genome. Toen hij terugkwam in Utrecht kreeg hij te horen dat hij nu binnen de Universiteit Utrecht een stage diende te doen terwijl hij net z'n zinnen had gezet op een buitenlands avontuur. Daarom werd een tweede belangrijke beslissing genomen en over te stappen naar Wageningen Universiteit. Terwijl deze moeilijke overstap werd geregeld werd ondertussen geld verdiend als medisch administratief assistent aan het USZO te Den Haag. In 1998 vervolgde hij zijn studie aan de Wageningen Universiteit. Eerst werd nog gedacht aan een stage in Australië maar er was ook contact met Dr. Nand Sibbing over mogelijkheden om de barbelen van het Tanameer in Ethiopië te gaan bestuderen. Hoewel er werd getwijfeld of dit nu wel zo verstandig was, je oom als begeleider, werd er besloten dat het avontuur werd aangegaan. Voor Arjan kwam de doorslag toen hij bezig was met z'n beslissing en hij in de treinkiosk het boek van Tijs Goldschmidt *Darwins Hofvijver* zag liggen dat een dergelijke problematiek beschrijft. Eerst werden nog enkele vakken gevolgd waarna hij in de zomer 1999 samen met zijn latere collega en paranimf Carlo Rutjes naar Ethiopië vertrok voor 6 maanden om daar onder supervisie van Dr. Nand Sibbing en Dr. Martin de Graaf de "Reproduction and ontogenetic transformations among the *Barbus* species flock of Lake Tana, Ethiopia" te bestuderen. Subsidies hiervoor had hij succesvol aangevraagd bij de Schuurman Schimmel – Van Outeren Stichting en het Fonds voor Onderzoek ten Behoeve van het Natuurbehoud (FONA). Hij bestudeerde tijdens deze 6 maanden de ontwikkeling van 4 soorten piscivore barbelen. Aangezien de kleinste exemplaren niet goed waren te identificeren werd besloten de paaigronden te zoeken om aldaar dieren te vangen en hom en kuit af strijken om zo jongen van bekende afkomst te kweken. Deze soorten paaiden af tijdens de regentijd in de kleien zijriviertjes van de Gumara rivier. Diverse soorten werden succesvol voortgeplant en opgekweekt in het lab. Tevens leken de diverse soorten in tijd en plaats van reproductie

gescheiden te zijn. Een interessant aanknopingspunt… Na terugkomst werd de stage succesvol afgesloten met een 9 en studeerde Arjan in 2000 af aan de Wageningen Universiteit. Als Ir vroeg hij succesvol beurzen aan bij het Schure-Beijerinck-Popping Fonds van de KNAW, het NWO en de Lucie Burgers Stichting en vertrok wederom 6 maanden naar Ethiopië om dit aanknopingspunt te bestuderen. Na terugkomst werden resultaten opgeschreven en gepubliceerd in het wetenschappelijk vakblad Animal Biologie met de foto's op de cover. In afwachting van een vervolg in de biologie werkte hij in 2001 als projectassistent bij het Projectbureau Inspectie Verkeer en Waterstaat. Carlo Rutjes bracht hem in contact met Dr. Guido van de Thillart die net van start zou gaan met een grootschalig EU project aan paling. Vanaf November 2001 tot Januari 2006 heeft hij als AIO de "Energetic reguirements and environmental constraints of reproductive migration and maturation of European silver eel (*Anguilla anguilla* L.)" bestudeerd resulterend in dit proefschrift en, tot nu toe, publicaties in Animal Biology, Aquaculture en Naturwissenschaften. Vanaf Januari 2006 is hij als onderzoeker binnen een door LNV gefinancierd project aangesteld om zwemmen als natuurlijke trigger van de maturatie bij paling te onderzoeken. In 2007 hoopt Arjan zijn weg te vervolgen in Barcelona of Valencia, in het geboorteland van zijn vriendin Eugenia.

De appel valt niet ver van de boom.

# DISSEMINATION

## 1. Scientific publications

1) Tanck,, M.W., Palstra, A.P., Weerd, M.V.D., Leffering, C.P., Poel, J.V.D., Bovenhuis, H., Komen, J. (2001). Segregation of microsatellite alleles and residual heterozygosity in homozygous androgenetic common carp (Cyprinus carpio L.). **Genome** 44 (5): 743-751.
2) Palstra, A.P., de Graaf, M., Sibbing, F.A. (2004). Riverine spawning and reproductive segregation in a lacustrine cyprinid species flock: facilitated by homing? **Animal Biology** 54 (4): 393-416. (Incl. Front pictures).
3) Palstra, A.P., Cohen, E., Niemantsverdriet, P., van Ginneken, V., van den Thillart, G.J.E.E.M. (2005) Artificial maturation and reproduction of European silver eel: Development of oocytes during final maturation. **Aquaculture** 249 (1-4): 533-547.
4) Ginneken, V. van, Vianen, G., Muusze, B., Palstra, A., Verschoor, L., Lugten, O., Onderwater, M., Schie, S. van, Niemantsverdriet, P., Heeswijk, R. van, Eding, E., Thillart, G. van den. (2005) Gonad development and spawning behaviour of artificially-matured European eel (Anguilla anguilla L.). **Animal Biology** 55 (3): 203-218. (Incl. Front pictures).
5) Palstra, A.P., van Ginneken, V.J.T., Murk, A.J., van den Thillart, G.E.E.J.M. (2006) Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? **Naturwissenschaften** 93: 145-148. (Short communication).
6) De Graaf, M., Nagelkerke, L.A.J., Palstra, A.P., Sibbing, F.A. Early morphological divergence and adaptive phenotypic plasticity among Lake Tana's *Labeobarbus* species flock (Cyprinidae): implications for their sympatric speciation. **Biological Journal of the Linnean Society**, submitted.
7) Palstra, A.P., Curiel, D., Fekkes, M., de Bakker, M., Székely, C., van Ginneken, V.J.T., van den Thillart, G.E.E.J.M. Swimming stimulates oocyte development of European eel (*Anguilla anguilla* L.). **Journal of Fish Biology**, submitted.
8) Ginneken, V. van, Palstra, A., Nieveen, M., Berg, H. van den, Hanzen, D., Lock, R., Spawnings, T., Niemantsverdriet, P., Thillart, G. van den, Murk, T. Effects of PCBs on the energy costs of migration and blood parameters of European silver eel (*Anguilla anguilla*). To be submitted.
9) Rankin, J.C., Palstra, A.P., Lafont, A.G., Fouchereau-Peyron, M., Madsen, S.S., van den Thillart, G.E.E.J.M. Acclimation to sea water in the European eel *Anguilla anguilla*: effects of silvering. To be submitted.
10) Palstra, A.P., van Ginneken, V.J.T., van den Thillart, G.E.E.J.M. Swim fitness of European silver eels (*Anguilla anguilla* L.). Journal of Experimental Biology, to be submitted.
11) Palstra, A.P., Heppener, D.F.M., van Ginneken, V.J.T., Székely, C., van den Thillart, G.E.E.J.M. Swim efficiency and reproductive migration of silver eels are severely impaired by the swim-bladder parasite *Anguillicola crassus*. Comparative Biochemistry and Physiology A – Molecular & Integrative Physiology, to be submitted.

12) Palstra, A.P., Antonissen, E., Clavero, M.E., Nieveen, M., Niemantsverdriet, P., van Ginneken, V.J.T., van den Thillart, G.E.E.J.M. The fate of fat in silver eels: lipid requirements for spawning migration. Marine Ecology Progress Series, to be submitted.

**2. Full papers and abstracts symposia**

13) Palstra, A.P., van Ginneken, V., van den Thillart, G., (2004). Artificial reproduction of the European silver eel (*Anguilla anguilla* L.). EAS Special Publication No 34.
14) Palstra, A.P., Cohen, E., Niemantsverdriet, P., van Ginneken, V., van den Thillart, G.J.E.E.M. (2004). Artificial maturation and reproduction of European silver eel: Development of oocytes during final maturation. International 5th symposium on fish endocrinology. September 5-9, 2004. Castellon, Spain.
15) Palstra, A., Dufour, S., Thillart, G. van den, Ginneken, V. van. (2005). Natural and artificial induction of maturation of European eel. Fish and Diadromy in Europe. Ecology, Management, Conservation. Bordeaux 29 March – 1 April 2005.
16) Thillart, G. van den, Palstra, A., Ginneken, V. van, Székely, C. (2005). Energetics of eel migration; swim fitness and swim capacity. Fish and Diadromy in Europe. Ecology, Management, Conservation. Bordeaux 29 March – 1 April 2005.
17) Ginneken, V. van, Thillart, G. van den, Palstra, A. (2005) Possible causes for the decline of the European eel population. Fish and Diadromy in Europe. Ecology, Management, Conservation. Bordeaux 29 March – 1 April 2005.
18) Palstra, A.P., Székely, C., van Ginneken, V., Thillart, G. van den (2005). Swim fitness of European eel (*Anguilla anguilla*). Comparitive Physiology and Biochemistry Part A: 141: S163-S173.
19) Rankin, C., Madsen, S., Lafont, A., Fouchereau-Peyron, M., Palstra, A., van den Thillart, G. (2006). Effects of silvering and maturation on acclimation to sea water in the European eel, Anguilla anguilla L. Comparitive Physiology and Biochemistry Abstracts SEB 2006 Canterbury.
20) G. van den Thillart, V. van Ginneken, A. Palstra (2006). The 6000 km spawning migration of the European eel. VIIth International Congress on the Biology of Fish. St John's, Newfoundland, Canada July 18-22.
21) Palstra, A.P., van Ginneken, V.J.T., Murk, A.J., van den Thillart, G.E.E.J.M. (2006). Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? ICES Annual Science Conference CM 2006/J: 34.

**3. Scientific publications for general public**

22) Palstra, A. (2002). De rovers van het Tanameer. De Roofvis 26: 58-63.
23) Palstra, A.P., Cohen, E., van Ginneken, V., van den Thillart, G. (2003). Succesvolle bevruchting en embryonale ontwikkeling van de Europese paling (Anguilla anguilla L.): Een nieuwe aanpak om de bevruchtingkansen te vergroten. Aquacultuur 18 (6): 18-21.
24) Palstra, A., Cohen, E., Clavero, E., Ginneken, V. van, Thillart, G. van den. (2005). Kunstmatige reproductie van Europese schieraal: Oocyt ontwikkeling tijdens

finale maturatie. In: CD-ROM Vissennetwerk 2004-2005. Publicatie Vismigratie. OVB eds.

25) Palstra, A. (2005). Een schier onmogelijke taak. De Roofvis 51: 52-57.

**4. Other publications**

26) Palstra, A. (2003). Passie per unit effort. De Karperwereld 23: 34-41.

27) Palstra, A. (2004). Buitenspel. De Karperwereld 36: 34-43.

28) Palstra, A. (2005). Gevonden in verloren uurtjes. De Roofvis, accepted.

**5. PhD thesis**

29) Palstra, A.P. (2006) Energetic requirements and environmental constraints of reproductive migration and maturation of European eel (*Anguilla anguilla* L.). PhD thesis. University of Leiden, the Netherlands.

**6. Publications in other PhD thesis's**

Tanck, M.W., Palstra, A.P., Weerd, M.V.D., Leffering, C.P., Poel, J.V.D., Bovenhuis, H., Komen, J. (2000). Segregation of microsatellite alleles and residual heterozygosity in homozygous androgenetic common carp (Cyprinus carpio L.). In: Selective breeding for stress response in common carp (*Cyprinus carpio* L.). PhD thesis. Wageningen Agricultural University, the Netherlands.

Palstra, A.P., de Graaf, M., Sibbing, F.A. (2003). Riverine spawning and reproductive segregation in a lacustrine cyprinid species flock: facilitated by homing? In: de Graaf, M. Lake Tana's piscivorous Barbus (Cyprinidae, Ethiopia): Ecology, Evolution, Exploitation. PhD thesis. Wageningen Agricultural University, the Netherlands.

De Graaf, M., Nagelkerke, L.A.J., Palstra, A.P., Sibbing, F.A. (2003). Early morphological divergence and phenotypic plasticity among Lake Tana's riverine spawning Barbus species: implications for their evolution. In: de Graaf, M. Lake Tana's piscivorous Barbus (Cyprinidae, Ethiopia): Ecology, Evolution, Exploitation. PhD thesis. Wageningen Agricultural University, the Netherlands.

Ginneken, V. van, Vianen, G., Muusze, B., Palstra, A., Verschoor, L., Lugten, O., Onderwater, M., Schie, S. van, Niemantsverdriet, P., Heeswijk, R. van, Eding, E., Thillart, G. van den. (2006) Gonad development and spawning behaviour of artificially-matured European eel (Anguilla anguilla L.). In: Ginneken, V. van. PhD thesis. Wageningen Agricultural University, the Netherlands.

Ginneken, V. van, Palstra, A., Nieveen, M., Berg, H. van den, Hanzen, D., Lock, R., Spawnings, T., Niemantsverdriet, P., Thillart, G. van den, Murk, T. (2006) Effects of PCBs on the energy costs of migration and blood parameters of European silver eel (*Anguilla anguilla*). In: Ginneken, V. van. PhD thesis. Wageningen Agricultural University, the Netherlands.

**7. Student thesis's**

Palstra, A.P. (1998). Genetical characterisation of androgenetic carp (*Cyprinus carpio* L.). Student thesis. Wageningen Agricultural University, the Netherlands.

Palstra, A.P. (2000). Reproduction and ontogenetic transformations among the *Barbus* species flock of Lake Tana, Ethiopia. Student thesis. Wageningen Agricultural University, the Netherlands.

**8. Invited lectures**

Leiden, The Netherlands, (October 30, 2003) Leiden University, section Integrative Zoology: The decisive phase in European eel's (Anguilla anguilla L.) life cycle: swimming * maturation * reproduction.

Nieuwegein, the Netherlands (January 12, 2004) OVB, oral presentation: Artificial reproduction of European eel: Clues for higher fertility rates from oocyte maturation.

Barcelona, Spain (May 2, 2005) Institut de Ciènces del Mar, Consejo Superior de Investigaciones Científicas tav Dr. F. Pifferer. Natural and artificial stimulation of maturation of European eel.

Valencia, Spain (May 5, 2005) Departemento de Ciencia Animal, Universidad Politéchnica de Valencia tav Dr. J. Asturiano. Natural and artificial stimulation of maturation of European eel.

Leiden, The Netherlands (March 21, 2006) Leiden University: This Week's Discoveries. Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama?

Leiden, The Netherlands (May 4, 2006) Leiden University for the course "Marine Biodiversity, Conservation and Management".

**9. Oral presentations**

Bordeaux, France (November 2002) EELREP 1st annual meeting: Swimming performance of European eel (*Anguilla anguilla* L.): A swimming fitness protocol.

Paris, France (November 12, 2003) EELREP maturation workshop: Artificial reproduction of European eel: Clues for higher fertility and hatching rates from individual oocyt maturation.

Leuven, Belgium (November 14, 2003) EELREP 2nd annual meeting: Swimming & maturation performance of European eel (*Anguilla anguilla* L.): Swimming fitness, long term simulated migration and hormonal stimulation.

Brest, France (July, 2004) EELREP 3rd annual meeting: Swimming and maturation performance of European eel.

Grietherbusch, Germany (December 16, 2004) oral presentation at the Rhine Silver Eel meeting: Natural triggers for reproductive migration and maturation of European eel.

Bordeaux, France (April 2005) DIADFISH oral presentation: Natural and artificial induction of maturation of European eel.

Barcelona, Spain (July 2005) Experimental Biology, oral presentation: Swim fitness of European eel (*Anguilla anguilla*).

Rome, Italy (January 2006), ICES/EIFAC working group on eels meeting, oral presentation: Estimation of the reproduction capacity of European eel (EELREP).

Maastricht, The Netherlands (September, 2006), ICES Annual Science Conference, oral presentation: Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama?

**10. Poster presentations**

Castellon, Spain (Sept. 2004) 5th int symp fish endocrinology poster presentation: Artificial reproduction of European silver eel: Development of oocytes during final maturation.

Barcelona, Spain (Oct. 2004) Aquaculture poster presentation: Artificial reproduction of European silver eel.

Maurik, the Netherlands (Nov. 2004) Vissennetwerk poster presentation: Artificial reproduction of European silver eel: Development of oocytes during final maturation.

**11. Active participation other symposia**

Pasaia, Basque country, Spain (October 2003) ICES/EIFAC working group on eels meeting, and cooperating in writing the yearly report.

Rome, Italy (January 2006) ICES/EIFAC working group on eels meeting, and cooperating in writing the yearly report.

Bilthoven, Netherlands (March 2006) Innovation Platform Aquaculture workshop on the recovery and the artificial reproduction of the eel.

**12. Scientific publicity**

New Scientist Magazine 2542 (09/032006) PCBs are killing off eels. p.6

**13. TV and newspaper publicity (2002- )**

TV interview (September 2002) for the program 'Iel' of Omrop Fryslân

Biological Newspaper Bionieuws 20, (28-11-2003): Paling plant zich voort in Leids laboratorium

Biological Newspaper Bionieuws 20, (28-11-2003): Zesduizend kilometer op een pakje boter

University Newspaper Mare (04/12/2003): Leidse aal laat zich kweken

Radio West (05/12/2003): Doorbraak met kweek van paling

Regional newspapers in Holland (06/12/2003): Doorbraak met kweek van paling

Regional Newspaper Haagsche Courant (06/12/2003): Leidse zoölogen melden doorbraak palingkweek.

Regional Newspaper Noordhollands Dagblad (08/12/2003): Doorbraakje in duistere wereld van de paling

Regional newspapers in Holland (May 2004): Doorbraak met kweek van paling

University Newspaper Leidraad (01/2004): Leidse aal laat zich kweken

National Newspaper Telegraaf (05/2004): Nederlandse biologen kweken palingembryo's

Regional Newspaper Goudse courant (05/2004): Paling in Leids lab gekweekt

TV Holland NOVA (05/04 and 08/2004): Broodje paling gered (20 min special on eel)

TV Germany (VOX; December 2004) 30 min broadcast of eel film made by Tesche

Radio Omroep Zeeland (28/1/2005) EVEX virus
National Newspaper Algemeen Dagblad (5/2/2005) Virus oorzaak van slechte palingstand
National Newspaper Trouw (6/2/2005) Palingen bezwijken aan virus
Radio KRO, interview van den Thillart (7/2/2005) Vroege vogels: Palingvirussen
National Newspaper De Volkskrant (26/11/2005) Het raadsel van de verdwijnende paling.
University Newsletter Leiden University (06/03/2006) Dioxines debet aan uitsterven paling
Radio Holland Centraal interview (10/03/2006) Dioxines debet aan uitsterven paling
Radio 1 VPRO (14/03/2006) Dioxines debet aan uitsterven paling
Biological Newspaper Bionieuws (May 2006) Dioxines frustreren palingembryologie
Magazine Grasduinen (due in May 2006) Dioxines debet aan uitsterven paling
Magazine Boomblad 18:2 (May 2006) Dioxines bedreigen paling
Magazine Fish Farming International 33 (5): 16-17 (May 2006) 'Only farming can save eels' says latest Dutch research
National newspaper Volkskrant (17/06/06) Zwemmen naar de Bermuda-driehoek in een aquarium
Biological Newspaper Bionieuws (June 2006) Dr. Dr. maar toch werkzoekend (interview met Vincent van Ginneken)
University magazine Origin 1:3 (July 2006) Hotel Atlantis
Radio West (14/07/2006) Waterspektakel
TV National Geographic (due in October)

**14. <u>Referee activities</u>**

Referee for General and Comparative Endocrinology since September 2005
Referee for Physiology & Behavior since February 2006
Referee for Animal Biology since February 2006

**15. <u>Reports</u>**

EELREP WP2 endreport "Swimming capacity of European eel from selected locations: Impact of environmental factors"
EELREP WP1 endreport participation "Silvering of European silver eel at selected locations"
EELREP WP4 endreport participation "Maturation performance of European eel from selected locations: Impact of environmental factors"
EELREP final report participation "Estimation of the reproduction capacity of European eel"
EELREP summary & recommendations participation "Estimation of the reproduction capacity of European eel"
ICES/EIFAC working group on eels report 2003 participation: chapter "Habitat Suitability"
ICES/EIFAC working group on eels report 2006 participation: chapter "Spawner Quality"
LNV report I "Swimming, natural trigger for eel maturation"

DISSEMINATION