Chapter 1

Introduction

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.

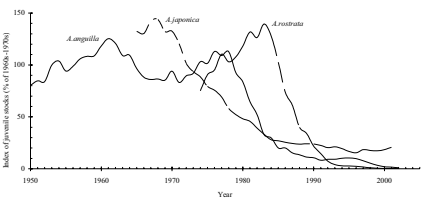

Figure 4 Time trends in juvenile abundance of the major eel stocks of the world (taken from anonymous, 2003).

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.

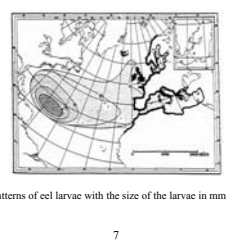

Figure 7 Distribution patterns of eel larvae with the size of the larvae in mm (Source: Schmidt 1923).

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.

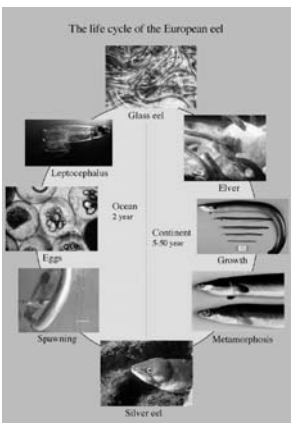

Figure 8 Life cycle of the European eel (taken from van Ginneken, 2006).

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).

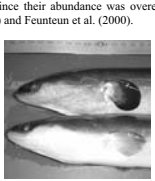

Figure 9 Two large eels (80 cm) from Lake Grevelingen. The eel on top has larger eyes, a darker bronze skin and dark pectoral fins. This eel is silver according to the eye index from Pankhurst (1982) and migratory (stage 4 according to Durif et al., 2005), the eel below is also silver according to the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

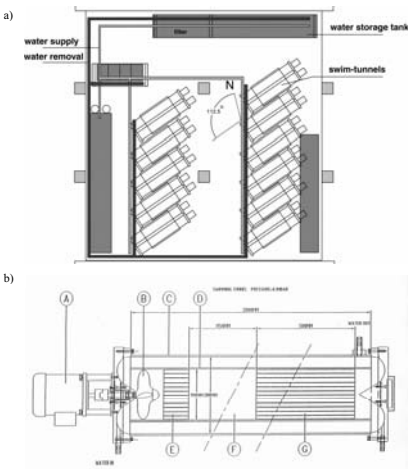

Figure 10 a) Swim-tunnel set-up (7000 l) of 22 127-l Blazka-type swim-tunnels placed in a WNW direction in a climatised 100 m^2 room and b) Schematic drawing of a 2.0-m swim-tunnel. The tunnel consists of 2 concentric Perspex tubes of two-meter and two PVC endcaps. A: electromotor, B: propeller, C: Perspex 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).

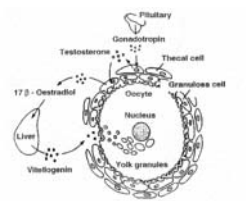

Figure 11 Part of the hypothalamo-pituitary-gonadal axis showing hormonal interactions between pituitary, gonads and liver.

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.

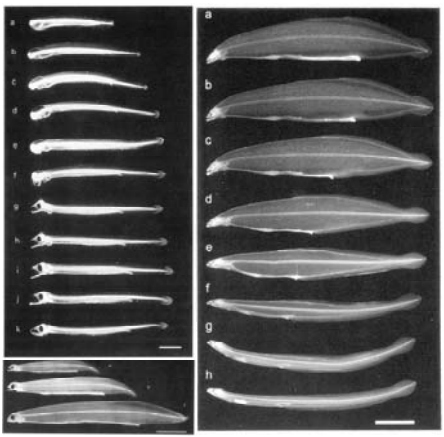

Figure 12 Captive-bred *A. japonica* preleptocephali, leptocephali and glass eels from hatching to 270 days after (taken from Tanaka, 2003).

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