Chapter 1
How to Estimate the Reproductive Success of European Silver Eels

Guido van den Thillart and Sylvie Dufour

1.1 Introduction

Over the past 25 years populations of the European eel have been declining to such a degree that major concerns have been raised for their long-term well being. Adult stocks started to dwindle in the 1940s in major areas of the continent, while recruitment (glass eel arrivals) has collapsed since the early 1980s. Fishing yield has gradually declined by 75% and the abundance of glass eels (young eel migrating from the ocean) is now below 5% of the historical level. The stock is considered outside safe biological limits, and immediate protection measures have been recommended. 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). EIFAC and related scientific work groups (Stone 2003) started a campaign to alert politicians, pointing to the rather serious collapse of the European and other eel populations. Also the EU-commission showed its concern by the fisheries policy publication of October 2003. Recently the EU has decided that all countries have to reduce the fishing pressure and to take protective measures such as allowing a 40% escapement of silver eels. Since 2007 the European eel is protected under CITES Appendix II (Annex B of Reg. (EC) 338/97). In addition the European Commission agreed in 2007 on measures for the recovery of the stock of European Eel (COM (2005) 472). Discussions have been going on for many years trying to pinpoint the

G. van den Thillart
Institute Biology, Leiden University, P.O. Box 9516, 2300 RA Leiden, The Netherlands

S. Dufour
Museum National d'Histoire Naturelle, DMPA, UMR CNRS 5178 "Biology of Marine Organisms and Ecosystems", 7 rue Cuvier, CP 32, 75231 Paris cedex 05, France

G. van den Thillart et al. (eds.), *Spawning Migration of the European Eel,*

causes that led to the downfall of a very common fish species. However, possibly due to the complex life cycle we have only indications such as: overfishing, restriction of habitat, migration barriers, and pollution.

The life cycle of the eel is fairly complex: (1) About 1 year as larvae drifting on the oceanic currents. (2) Then a few months as glass eels swimming into the estuaries along the European coasts. (3) This is followed by the longest interval: a feeding interval of 4 to 20 years as yellow eels. (4) Then, finally a second metamorphosis from yellow into the silver form takes place. This last interval has a duration of about 6 months, which includes downstream migration and an unknown oceanic interval of long distance migration to the spawning sites. Although the natural spawning process has never been observed, circumstantial evidence suggests that spawning takes place in the Sargasso Sea, some 6,000-km away (Miller and McCleave 1994; Schmidt 1923; Tesch and Wegner 1990). Further proliferation of the gonads must take place during the migration and/or during their short stay at the spawning site. A few records indeed show a GSI (gonad somatic index) higher than 10 for ocean caught silver eels (Bast and Klinkhardt 1988). The life cycle of the migrating silver eel can thus be characterized by refusal of food; an impressive swimming activity; and final maturation of the gonads.

During the second metamorphosis or ‘silvering’, eels go through a number of morphological and physiological changes which prepares them for their oceanic migration. All sensory organs become more developed: eyes and nostrils are enlarged, the lateral line is more visible (Barni et al. 1985; Dave et al. 1974; Lewander et al. 1974; Pankhurst and Lythgoe 1983). At the silver stage, eels stop feeding (Fricke and Kaese 1995; Tesch 1977) and their digestive tract regresses, the structure and metabolism of the liver changes too (Hara et al. 1980). Moreover, silver eels are sexually more developed than yellow eels, although the gonads remain in a pre-pubertal stage (Dufour et al. 2003). The silvering process is a complex phenomenon. The actual sequence of events (intermediate phases), the link between external and internal modifications as well as the duration of the silvering process, remain largely unknown. Up to now, the identification of silver eels was generally based on Pankhurst’s eye index. An eye index value of 6.5 was supposedly the threshold between yellow and silver eels. But even though the eye index correlates with GSI, a large sized eel may have a high eye index without necessarily being a migrant. Moreover, the 6.5 limit was based on artificially matured individuals and no direct link to downstream migration was made. An eye index of 6.5 therefore overestimates the number of migrants. Recently a detailed description of the silvering process was conducted on a subpopulation of eels from the Grand-Lieu Lake in France. From this five stages (F-I to F-V) for females and two stages for males were based on four easy to measure external parameters (length, weight, eye diameter, and pectoral fin length), predicting the stage with an accuracy of 82% (Durif et al. 2005). The pectoral fin length increases significantly when eels start their downstream migration. These fins do not actually propel the eel in the water, but they contribute to its stabilization in the open water and this may explain the increase in length at the time of migration.

1.1.1 Possible Causes for the Decline

So far, the causes for the decline of the eel population are not known, we have only assumptions without hard evidence. Apart from the most obvious factors such as habitat reduction, and overfishing, also pollution, in particular with PCB's (De Boer et al. 1999), infections with the swimbladder parasite (*Aguillicola crassus*) and the EVEX virus (Van Ginneken et al. 2004) might be causes for the current decline of the eel population. PCB's accumulate in the fat tissue of eels during their feeding interval (yellow eels), even to levels that make them by totally unsuited for human consumption (Dirksen et al. 1995; Reijnders 1986). During their spawning migration the silver eels do not eat, instead they use their fat for swimming. As a result of increasing PCB plasma levels, the animals may not be able to reach their spawning ground. It is likely that PCBs interfere with hormonal regulation and/or gonad development. Furthermore, not only will the adult eels be directly affected by the toxicity of PCBs but also subsequent fertilisation (Spies et al. 1988), hatching (Hose et al. 1982; Von Westernhagen et al. 1987) and viability of the larvae (Von Westernhagen et al. 1981) will be disturbed by high PCB levels. It is unknown to what extent levels of PCBs rise in blood plasma, fat and gonads in migrating silver eels during their journey to the Sargasso Sea, but they may well reach toxic levels. A large proportion of the European eel population is seriously infected with the swimbladder parasite (Moravec et al. 1994; Thomas and Ollevier 1992) and with the EVEX virus (Van Ginneken et al. 2004). Both have been introduced recently in Europe from Asia and are candidates for the rapid decline of the eel populations. Eels infected with the swimbladder parasite have a lower cruising speed (Barni et al. 1985). This may have serious consequences for the adult silver eels when migrating to the spawning grounds. In addition, infected eels loose the functionality of their swimbladder (Haenen 1995; Kleckner 1980), which means that they cannot maintain buoyancy and thus lose depth orientation.

1.1.2 Artificial Reproduction

Maturation and artificial reproduction of eels has been studied since the early work of Fontaine (1936). In Japan this research has been continued since the 1970s, resulting in fertilized eggs and non-feeding larvae in 1997 (Ohta et al.). Research on the induction of maturation and larval rearing of the Japanese eel, *Anguilla japonica*, is being conducted in mainland China and Japan. Most progress has been made at the National Research Institute of Aquaculture (Nansei, Japan). The maturation procedure involves injection of salmon pituitary extract (20 mg per week) for 11 or 12 weeks. At 24-h after the last injection, 17, 20 β-dihydroxy-4-pregnen-3-one was injected (2 μg g^{-1} body weight) to induce ovulation within 15–18 h. Five or six weekly injections of human chorionic gonadotropin (1 IU g^{-1} body weight) induced spermiation in male eels. Up to 62.8% fertility and 54.3% hatchability were obtained in the best case. (Ohta et al. 1997). Currently maturation and fertilisation result in controlled hatching and most larvae commence feeding. Also since 2001 leptocephali from *Anguilla japonica* were reared through metamorphosis, resulting in several yellow eels. This suggests that the eel life cycle may be closed in the near future. The artificially produced larvae are, however, very much smaller than wild leptocephali, when they arrive at the coast of Taiwan (Fricke and Tsukamoto 1998). Despite the enormous investments in time and energy, the Japanese scientists did not fully resolve the basic and applied question of the reproduction of eels. Obviously this stage is not reached with the European eel; only recently some papers have appeared showing successful fertilisation and embryonic development, but hatched larvae die before feeding (Palstra et al. 2005; Pedersen 2004). The fact that many fertilized eggs don't develop and that most larvae die prematurely, indicate that the problems may lie in the quality of eggs and possibly also in the quality of sperm. We assume that a major aspect of this problem may be resolved by trying to find natural stimuli of maturation and reproduction.

1.1.3 Maturation Sensitivity of Wild Eels

For wild silver eels, recruitment depends apart from successful migration also on successful spawning. As both processes occur in the ocean, we need other parameters to estimate reproductive success of the migrating silver eels. Thus far, there are no indicators for recruitment success. While obviously quality is of major importance, only the numbers of escaping silver eels are currently used in protection measures. A possible quality parameter is the sensitivity of silver eels to hormonal stimulation.

Full gonadal development has been successfully and repeatedly induced in female and male silver European eels, using the classical gonadotropic treatments (CPE- carp pituitary extract -in females and hCG- human chorionic gonadotropin -in males). As demonstrated by the ability of exogenous gonadotropic treatment to induce maturation, the insufficient production of endogenous gonadotropins is responsible for the blockade of eel maturation. Eel LH (luteinizing hormone) and FSH (follicle-stimulating hormone) are known to be expressed at a low level in the pituitary of silver eels. These hormones are regulated in an opposite manner during induced maturation, with a large increase in LH synthesis, but a decrease in FSH, likely resulting from differential endogenous steroid feedbacks. It has been shown that LH release is under a dual neurohormonal control, positive by GnRH (gonadotropin releasing hormone) and negative by dopamine.

High variability in the kinetics of oocyte maturation and egg quality has been observed. Maturation performance of female silver eels, as estimated by their gonadal development in response to short term and long term CPE treatments, correlates to individual initial biometric parameters. Inter-individual variability of ovarian development in response to gonadotropic treatment has often been mentioned, but this question was never directly addressed. Clearly it would be very useful if we would be able to predict which silver eel respond quickly and which would respond slowly or not at all.

1.2 Long Term Swimming

An important aspect of the reproduction of European silver eels is the huge distance they have to swim to reach their spawning grounds. After leaving the West European coast they still have to swim 5,000–6,000 km to the Sargasso Sea, the assumed spawning site. To cover this distance female eels must swim continuously for 6 months at 0.5 BL/s, which requires an impressive endurance as well as high energy reserves coupled with low cost of transport. So, obviously long term swimming capacity is a major prerequisite for reproduction. Until recently no long term swimming studies were carried out. It was not known how much energy is required for the crossing of the Atlantic Ocean. Still, this knowledge is crucial for any estimation about recruitment. We need to know at what speed eels swim, whether they can swim continuously, and at what energy costs. The energy requirement of course corresponds fat content, obviously eels must be fat to cross the ocean, but how fat?

The capacity to swim across the Atlantic Ocean can easily be influenced by several environmental factors such as parasites, virus infections, and pollutants. The swimbladder parasite *A. crassus* is known to have infected all European eel populations in the 1980s. Although eels usually don't die from the infection, it affects buoyancy control and therefore swimming mode and navigation in open water. Similarly infection with the eel viruses is not lethal, but those infections might be more severe in combination with strenuous swimming for a long period.

1.2.1 High Pressure

Also very impressive is the requirement of silver eels to navigate at 50–200 atm water pressure. Current knowledge suggests that pressure is an important factor because of its disturbing effects on biophysical and biochemical processes (Kleckner 1980). Previous experiments have shown that the first hours under pressure constitute a critical period during which the mortality could be high (Sebert and Barthelemy 1985). This mortality is related to the fish's fitness. However, pressure has also been suggested as a stimulus for maturation (Fontaine et al. 1985).

1.2.2 Our Goal

This book has been written for only one reason: to provide information about the quality of escaping silver eels by estimating the chance of success in reproduction. The current state of knowledge is such that we can make reasonable estimates of the escaping silver eels for reproduction. That implies that new ways for eel conservation can be implemented; instead of only setting percentages of escapement, it would be wise to include also the protection of habitats that produce high quality spawners. A second implication of the current knowledge is that this knowledge should help us to find solutions for artificial reproduction. The current problem of eel reproduction is the limited fertility of adults and the large scale mortality of larvae; understanding the natural process of silvering and maturation should be understood in order to develop successful protocols.

Acknowledgements This work has been supported by the European contract EELREP N°Q5RS-2001-01836.

References

Barni S, Bernocchi G, Gerzeli G (1985) Morphohistochemical changes during the life cycle of the European eel. Tissue Cell 17: 97–109.

Bast HD, Klinkhardt MD (1988) Fang eines Silberaales (Anguilla anguilla L. 1758) im Iberischen Becken (Nordost Atlantik) (Teleostei: Anguillidae). Zool. Anz. 221: 386–398.

Castonguay M, Hodson PV, Moriarty C, Drinkwater KF, Jessop BM (1994) Is there a role of ocean environment in American and European eel decline? Fish. Oceanogr. 3(3): 197–203.

Dave G, Johansson ML, Larsson A, Lewander K, Lidman U (1974) Metabolic and haemotological studies on the yellow and silver phases of the European eel, Anguilla anguilla L.-11. Fatty acid composition. Comp. Biochem. Physiol. 47B: 583–591.

De Boer J, Pieters H, De Wit M (1999) Verontreinigingen in aal en snoekbaars: Monitorprogramma ten behoeve van de Nederlandse sportvisserij, 1998. (RIVO-DLO report C036/99, The Netherlands).

Dirksen S, Boudewij TJ, Slager LK, Mes RG, Van Schaick MJM, De Voogt P (1995) Reduced breeding success of cormorants (*Phalacrocorax carbo sinensis*) in relation to persistent organochlorine pollution of aquatic habitats in the Netherlands. Environ. Pollut. 88: 119–132.

Dufour S, Burzawa-Gerard E, Le Belle N, Sbaihi M, Vidal B (2003) Reproductive endocrinology of the European eel, Anguilla anguilla. In: Aida K, Tsukamoto K, Yamauchi K (eds) Eel Biology. Springer, Tokyo, pp. 373–386.

Durif C, Dufour S, Elie P (2005) The silvering process of Anguilla anguilla: A new classification from the yellow resident to the silver migrating stage. J. Fish Biol. 66: 1025–1043.

Fontaine M (1936) Sur la maturation complete des organes genitaux de l'anguille et l'emission spontane des produits sexuels. C.R. Acad. Sci. Paris, 252: 1258–1260.

Fontaine YA, Dufour S, Alinat J, Fontaine M (1985) A long immersion in deep sea stimulates the pituitary gonadotropic function of the female European eel (Anguilla anguilla L.). C. R. Acad. Sci. Paris, Ser. III 300: 83–87.

Fricke H, Kaese R (1995) Tracking of artificially matured eels (*Anguilla anguilla*) in the Sargasso Sea and the problem of the Eel's Spawning Site. Naturwissenschaften 82: 32–36.

Fricke H, Tsukamoto K (1998) Seamounts and the mystery of eel spawning. Naturwissenschaften 85: 290–291.

Haenen OLM (1995) *Anguillicola crassus* (Nematoda, Dracunculoidea) infections of European eel (*Anguilla anguilla*) in the Netherlands: Epidemiology, pathogenesis, and pathobiology. Ph.D. thesis. Agricultural University, Wageningen, 127 pp.

Hara A, Yamauchi K, Hirai H (1980) Studies on female specific serum protein (vitellogenin) and egg yolk protein in Japanese eel (*Anguilla japonica*). Comp. Biochem. Physiol. 65B: 315–320.

Hose JE, Hannah JB, DiJulio D, Landolt ML, Miller BS, Iwaoka WT, Felton SP (1982) Effects of benzo(a)pyrene on early development of flatfish. Arch. Environ. Contam. Toxicol. 11: 167–171.

Kleckner RC (1980) Swimbladder volume maintenance related to initial migratory depth in silver-phase Anguilla rostrata. Science 208: 1481–1482.

Lewander K, Dave G, Johansson ML, Larsson A, Lidman U (1974) Metabolic and haemotological studies on the yellow and silver phases of the European eel, *Anguilla anguilla* L.-I. Carbohydrate, lipid, protein and inorganic ion metabolism. Comp. Biochem. Physiol. 47B: 571–481.

Miller M, McCleave JD (1994) Species assemblages of leptocephali in the subtropical convergence zone of the Sargasso Sea. J. Mar. Res. 52: 743–772.

Moravec F, Di Cave D, Orecchia P, Paggi L (1994) Experimental observation on the development of *Anguillicola crassus* (Nematoda: Drancunuloidea) in its definitive host, *Anguilla anguilla* (Pisces). Folia Parasit. 41: 138–148.

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

Palstra AP, Cohen EGH, Niemantsverdriet PRW, Van Ginneken VJT, Van den Thillart GEEJM (2005) Artificial maturation and reproduction of European silver eel: Development of oocytes during final maturation. Aquaculture 249: 533–547.

Pankhurst NW, Lythgoe JN (1983) Changes in vision and olfaction during sexual maturation in the European eel *Anguilla anguilla* (L.). J. Fish Biol. 23: 229–240.

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

Reijnders PJH (1986) Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324: 456–457.

Schmidt J (1923) Breeding places and migration of the eel. Nature 111: 51–54.

Sebert P, Barthelemy L (1985) Effects of high hydrostatic pressure per se, 101 atm, on eel metabolism. Resp. Physiol. 62: 349–357.

Spies RB, Rice DW, Felton JH (1988) Effects of organic contaminants on reproduction of the starry flounder *Platichhys stellatus*, in San Francisco Bay 11. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. Mar. Biol. 98: 191–200.

Stone R (2003) Freshwater eels are slip-sliding away. Science 302: 221–222.

Tesch FW, Wegner G (1990) The distribution of small larvae of Anguilla sp. related to hydrographic conditions 1981 between Bermuda and Puerto Rico. Int. Rev. Ges. Hydrobiol. 75: 845–858.

Tesch FW (1977) The eel, Biology and Management of Anguilled Eels, Chapman & Hall, London, 434 pp.

Thomas K, Ollevier F (1992) Population biology of *Anguillicola crassus* in the final host *Anguilla anguilla*. Dis. Aquat. Org. 14: 163–170.

Van Ginneken V, Haenen O, Coldenhoff K, Willemze R, Antonissen E, Van Tulden P, Dijkstra S, Wagenaar F, Van den Thillart G (2004) Presence of virus infections in eel species from various geographic regions. Bull. Eur. Ass. Fish Pathol. 24(5): 268–272.

Von Westernhagen, H., Rosenthal, H., Dethlefsen, V., Ernst, W., Harms, U.L., Hanse, P.D., Cameron, P., Janssen, D. 1981. Bioaccumulating substances and reproductive success in Baltic flounder *Platichthys flesus. Aquatic Toxicology*, 1: 85–99.

Von Westernhagen H, Dethlefsen V, Cameron P, Janssen D (1987) Chlorinated hydrocarbon residues in gonads of marine fish and effects on reproduction. Sarsia 72: 419–422.