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EVALUATION OF SILVER EUROPEAN EEL (*ANGUILLA ANGUILLA*) FOR THE IMPLEMENTATION OF AN EFFECTIVE EEL MANAGEMENT PLAN IN MEDITERRANEAN COASTAL LAGOONS.

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Evaluation of silver European eel (*Anguilla anguilla*) for the implementation of an effective Eel Management Plan in Mediterranean coastal lagoons.

PhD Thesis, 2012 – University of Bologna, Italy.

Summary

Glossary

AChE acetylcholinesterase
ASCh acetylthiocholine iodide
ASR adequate sampling ratio
BChE butyryl-cholinesterase
CF condition factor
EI eye index
ED eye diameter
EDh horizontal eye diameter
EDv vertical eye diameter
FL pectoral fin length
GSI gonado-somatic index
HSI hepato-somatic index
HT hematocrit
L total body length
MD mean eye diameter
OP otolith polisher
OTO age reading by otolith
PChE propionylcholinesterase
PCV packed blood red cell volume
RBC Red blood cell
SCA age reading by fish scale
SE skin evaluation
SEELF Sustainable EEL Fishery
SI silver index
W total body weight
WBC white blood cell
WG gonads weight
WL liver weight

1. Introduction

A female European eel captured in Comacchio, 1960s *circa*.

1.1 Decline of the European eel

The European eel (*Anguilla anguilla*) is a catadromous fish species present across the European continent and along the Mediterranean coasts (Tesch, 2003). The life-history of this fish depends strongly on oceanic conditions; maturation, migration, spawning, larval transport and recruitment dynamics are completed in the open ocean (Tesch, 2003). Its spawning grounds thousands of kilometers away in the ocean, possibly the Sargasso Sea (Knights, 1996; Feunteun, 2002; Aarestrup *et al.*, Science 2009). Leptocephalus larvae are transported along the Gulf Stream and North-Atlantic Drift for a journey of seven to eleven months (Lecomte-Finiger, 1992) or some years (Schmidt, 1922; Liew, 1974; Boetius and Harding, 1985; van Utrecht and Holleboom, 1985; Feunteun, 2002). The continental distribution of the European eel includes virtually all types of waterbodies, namely rivers, lakes, reservoirs, coastal lagoons, estuaries and coastal areas (Moriarty & Dekker, 1997).

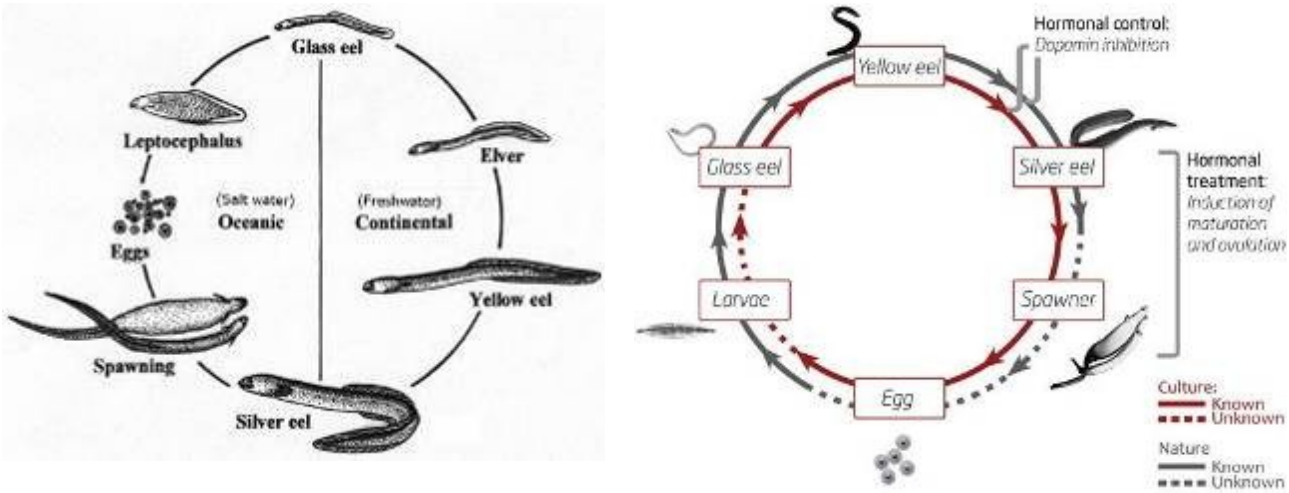


Figure 1.1.1. Biological cycle of European eel (left, ICES) and its knowledge in nature and culture (right, pro-eel.eu)

In the past three decades, European Eel has suffered an intensive decline in the major part of its distribution area, and the stocks are now considered to be below safe biological limits (Moriarty and Dekker, 1997; Feunteun, 2002; Dekker, 2004; ICES 2010). This decline was reported by scientists in the 1940s in Northern Europe, and again in the 1980s across the rest of its continental range. Recruitment of glass eel decreased significantly in the early 1980s, and it recently dropped to 1% of the levels encountered in the 1970s (ICES, 2002) and stocks are now considered to be below safe biological limits (Moriarty and Dekker 1997; Feunteun 2002; Dekker, 2004; ICES, 2010).

Figure 1.1.2. Picture of European eel in natural environment (ARKive, © Tim Martin / naturepl.com).

The reasons for these declines are not well understood and different factors are likely to have contributed to such decline, including overfishing, habitat loss, presence of parasites, climate change, and poor water quality mainly related to chemical pollution (Feunteun, 2002). The impact of these factors on eel populations is exacerbated by the complex biological cycle of the fish which includes an extremely long migration in marine waters. Adults die after spawning, while larvae will return along the coastal waters and newly metamorphosed glass eel will migrate upstream into estuarine and fresh waters. The growth phase (yellow eel) in continental waters lasts for several years (6-12 for males and 9-20 for females), and ends with a second metamorphosis called silvering (silver eel), that immediately precedes the transoceanic reproductive migration (Colombo and Grandi, 1995). The transformation from yellow to silver eels is therefore a key event preparing the future spawners for migration and reproduction. Modifications occur in association with hormonal surges and are linked to the transition between fresh and salt water, the beginning of the sexual maturation, and the preparation of the swimming activity which is exhausting in terms of energy resources (Palstra et al., 2010). During their continental life cycle phase, eels accumulate a considerable amount of lipid reserves, which are fundamental for the success of the long oceanic migration since during this period they do not feed (Robinet and Feunteun 2002; Ribeiro et al. 2005; Palstra et al. 2006) and their digestive tract regresses (Durif et al. 2005; van Ginneken et al. 2007).

Additional causes of the stock decline are reviewed by several authors (i.e. Dekker, 2003; Knights, 2003; Tapie et al., 2011), and address global change as a main factor. Indeed global change is thought to provoke a northwards deviation of Gulf Stream currents (e.g. Knights et al., 1996), which makes the transoceanic migration back to the European coasts much longer or even impossible.

At the continental level, exploitation, habitat loss, migration route obstruction, and transfer

of parasites and diseases may contribute to the decline. Finally, poor condition and lower energy reserves were reported to interfere with the migration of silver eels from the European coasts to the Sargasso Sea and hamper successful reproduction (Larsson et al., 1990; van Ginneken et al., 2007).

Exposure to pollution has been indicated as one of the causes that may have been contributing to the decrease of the populations of this species (Feunteun, 2002; Robinet and Feunteun, 2002), particularly during the continental phase of its life cycle when animals may spend several years in close contact with contaminated sediments. Pollutants may directly interfere with the evolution of eel populations by several ways, for example: through the impairment of functions determinant for the survival and performance of the organisms and increasing the mortality ratio in the population; by delaying the development, thus, interfering with the generation time and decreasing the population renewal; and/or by decreasing the health condition of the individuals reducing the probability of a successful migration to the reproduction area.

Figure 1.1.3 A glass eel of *A. anguilla*, estimated age 2 years, captured in Italy (Courtesy of ARPA Ferrara).

Furthermore, pollution can also have indirect adverse effects on eel populations by for example decreasing the water quality (e.g. eutrophication) or decreasing the availability of food by reducing prey populations. Eel are efficient bioaccumulators of xenobiotics as a result of their high fat content, long life cycle, and exposure to contaminated sediments. Although there is no proof of significant mortality due to persistent pollutants, accumulated tissue xenobiotics may be released at the time eel use fats to support swimming and gonad maturation. The accumulated chemicals may be released into the blood due to fat mobilization during the long migration to the Sargasso Sea causing acute or chronic toxic effects that may include a decrease of eel's reproductive capability.

Pesticides probably disturb fat accumulation and/or provoke non physiological lipid mobilization in eels, possibly through acethylcholinesterase inhibition, generating

involuntary and continuous muscular activity (Sancho et al., 1998). Gimeno et al. (1995) also reported that pesticide exposure decreased glycogen and increased lactate in muscle, and induced hyperglycemia thus increasing consumption of energetic resources. Eels do not eat during their migration (up to 6,000 km from the European sea coasts to the Sargasso Sea), and it is therefore assumed that they had previously accumulated sufficient energy reserves. Furthermore, the starving eels provide for their energy demand by using fat tissue. Lipophilic chemicals stored in the fat will then be released into the blood plasma, making them available for toxicity. Therefore, increasingly high plasma levels of xenobiotics including pesticides could make the eel fail in its attempt to reach the spawning grounds (van Ginneken et al., 2009).

The European eel was included in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (CITES, 2008) and recognized as an “endangered species”. European eel restoration was attempted over the last decades (Moriarty and Dekker, 1997) but the decline continues.

The European Union with Regulation n.1100/2007 imposed monitoring and restocking of this species as defined in an Eel Management Plan (EMP; EC, 2007) for each river basin. The Regulation states that the 40% of the silver eel biomass escaping from inland waters must reach the sea in order to increase the possibility to restore naturally European eel populations. However, no tools for the evaluation of escaping fish were provided within the EMP, where the quality of breeders, among other parameters, may represent a valuable target within a restoration program.

As discussed by Geeraerts and Belpaire (2010), the EU eel recovery plan (EC, 2007) should include evaluation of silver eels (e.g. contamination levels, biomarker responses, lipid content and condition) as well as a comprehensive overview of the quality of the silver eel population all over Europe seems to be an essential and urgent objective for the European eel management.

1.1.1 Research topics for a fast restoration of European stock: pros

The fast restoration of eel population at continental level, is possible considering a multidisciplinary approach on fish physiology, use of biomarkers, habitats restoration and conservation, environmental management.

This multidisciplinary approach is discussed at least by two recent initiatives, as EELIAD Project and SEG-Sustainable Eel Group.

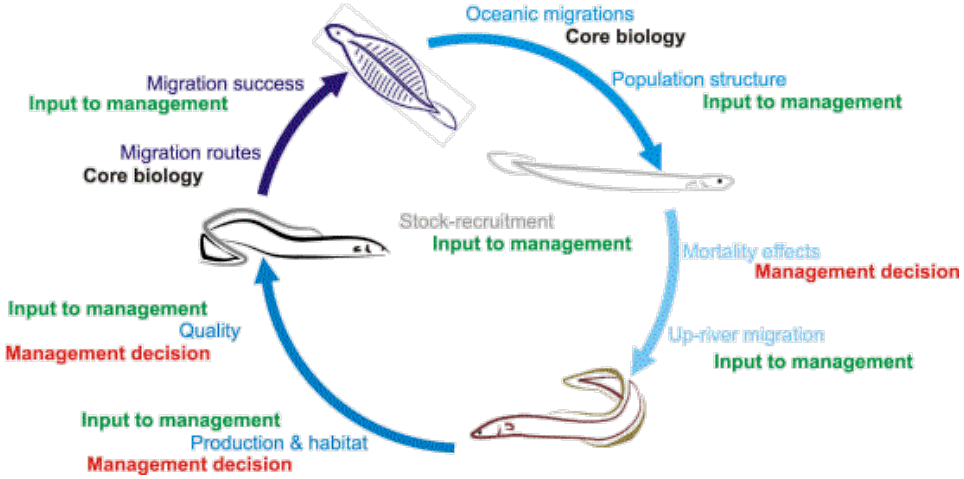


Figure 1.1.4. Evaluation of biological cycle of European eel and key factors for a sustainable eel management (www.eeliad.com).

More concretely, the Sustainable Eel Group is formed by scientists, producers and conservationists interested into the conservation of European eel; SEG argue that the fish production have to be part of the conservation strategy of *Anguilla anguilla*, as proved in some area, like Comacchio lagoon.

The Eel paradox is that *we have to eat an endangered species in order to protect it*. This paradox is produced by experience: the biodiversity cannot be protected exclusively because of its natural value. In order to improve the capability to act effectively in short time, SEG aims to involve commercial topics that support the restoration of European eel stock. In this way, media and decision makers (politicians as well) pay more attention.

Sustainable *Eel* Group

Figure 1.1.5. The logo of the Sustainable Eel Group

The Sustainable Eel Group is launching a certification of “sustainable eel” and “sustainable eel product”:

CERTIFIED SUSTAINABLE EEL ™ CERTIFIED SUSTAINABLE EEL

CERTIFIED SUSTAINABLE EEL PRODUCT ™ CERTIFIED SUSTAINABLE EEL PRODUCT

Figure 1.1.6. SEG’s logos for certified “sustainable eel” and “sustainable eel product”. (Logos are ™ of SEG)

SEG logos aims to ensure that any eel or eel product, which meets the SEG standard or has on of these logos affixed to it, has been produced sustainably using the best practices and the best available science. Before any eel or eel product can be labelled using one of the SEG logos a business must be independently audited by a SEG approved auditor. It is necessary for each and every business in the supply chain to be audited by independent auditors approved by SEG to ensure the provenance and chain of custody of the product. Each business, once having been approved by the auditor, will be required to sign a license agreement with SEG to enable them to use the logo or place the logo on to any product.

1.1.2 Research topics for a fast restoration of European stock: cons

Currently some research topics are largely presented as bottle-neck for the restoration of stock of European eel: a) panmixia and migration routes, b) reproduction under controlled conditions, c) parasite infestation and barriers to migration.

A. Panmixia and migration routes

Eels are catadromous fishes that spawn in tropical ocean waters, and constitute a single genus Anguilla (Schrank, 1798) with a peculiar leptocephalus larval stage. As a whole, different species of eels are extremely similar in morphology (Teng et al., 2009). Although the phylogenetic synopsis (Ege, 1939) for the genus Anguilla was widely accepted, recently Lin et al. (2005) states that appropriate methods for evaluation of morphological data (fig. 1.1.7 right) can give similar phylogenetic trees for freshwater eels as do molecular data (fig. 1.1.7 left). Atlantic eels, *A. anguilla* and *A. rostrata*, are near in both representation reported in figure 1.1.7, as well as they are geographically separated from other species in the Pacific and Indian Oceans. This topic could be of interest in restocking of European eel, because of possible mixing of the two species. Panmixia is widely accepted (van Ginneken et al, 2005) and Aarestrup et al. (2009) have recently confirmed that adults migrate to Sargasso Sea following the oceanic currents (see figure 1.1.8 and 1.1.9), as previously argued by others (Tesch, 2003). Although the European eel (*Anguilla anguilla*) is considered a single homogeneous population (Vasemagi, 2009), detailed evidences of panmixia is an interesting topic for the scope of this work. Future research on eel reproductive migration could benefit from fish tagging and device as WaveGliders (Cornell University, figure 1.1.10): this instrument would be released off the coast and operate along survey lines remotely controlled by scientists on shore.

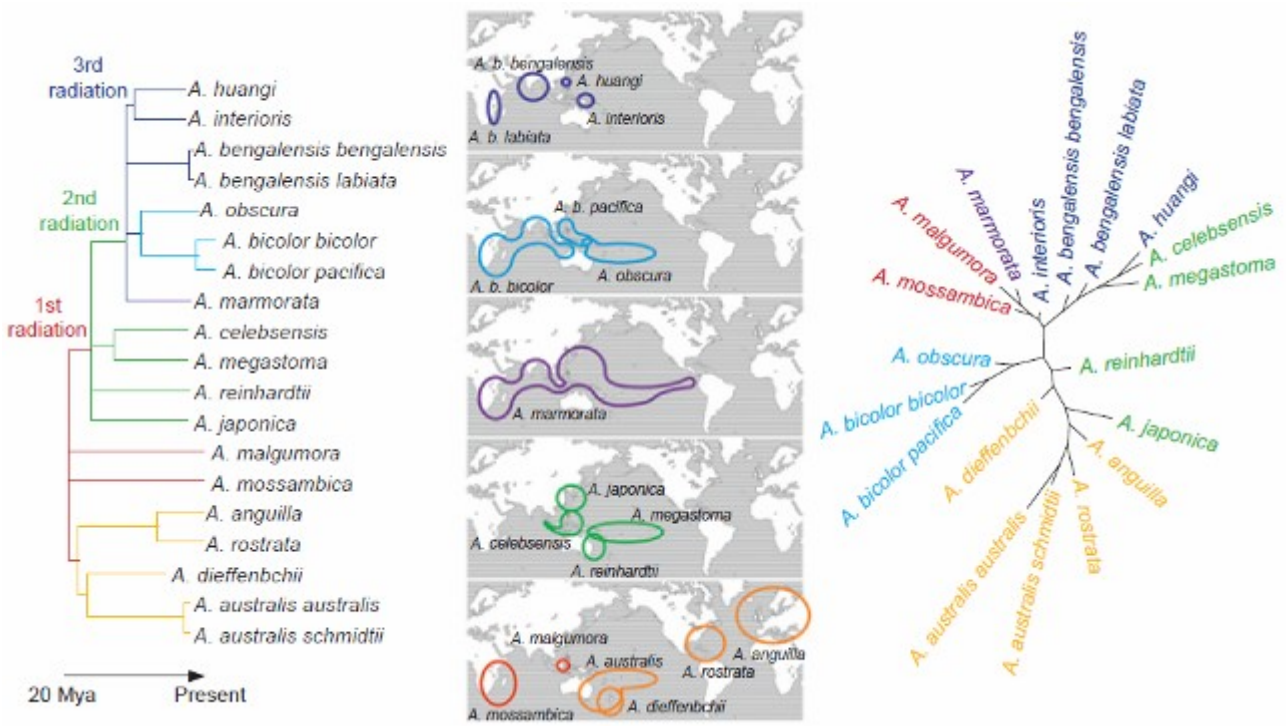


Figure 1.1.7. The genus *Anguilla*: an inferred molecular phylogeny based on 13 mitochondrial protein coding sequences (left), the current distribution (center) and a morphological phylogenetic tree. (Teng et al., 2009).

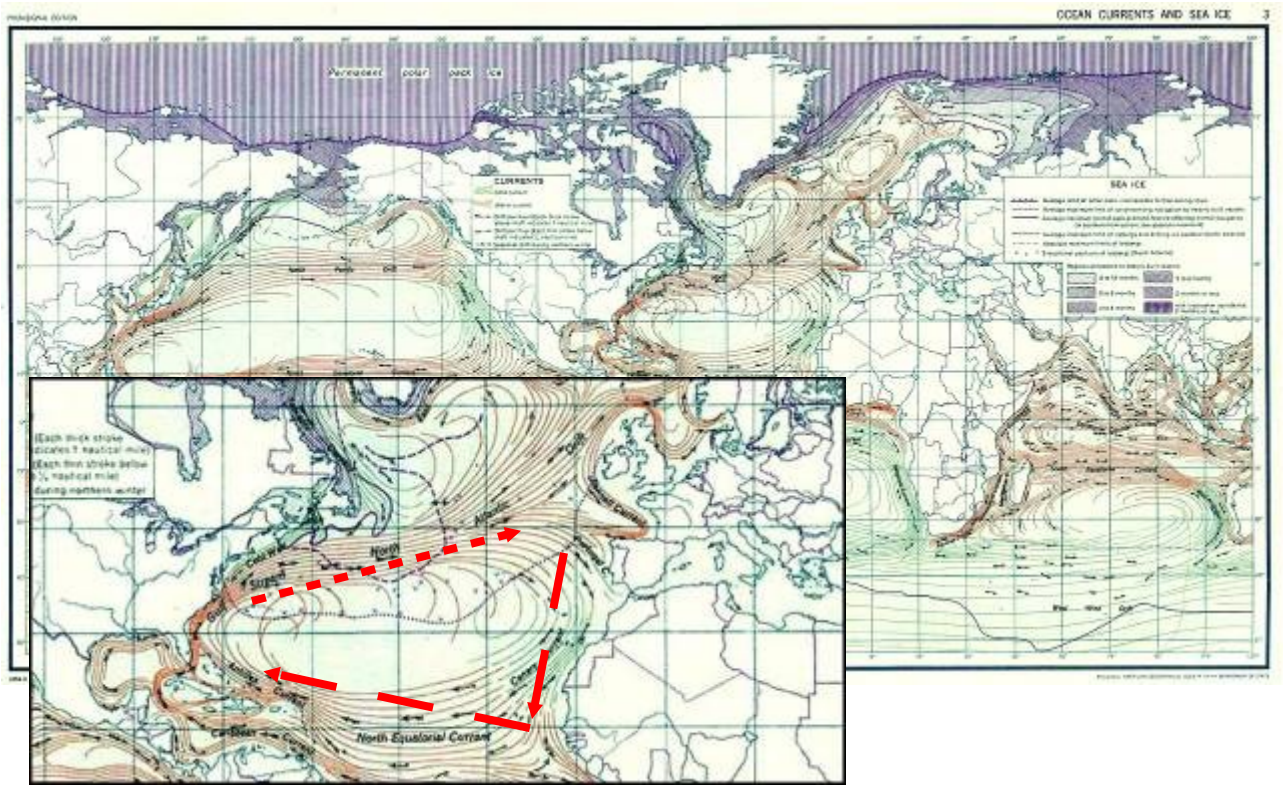
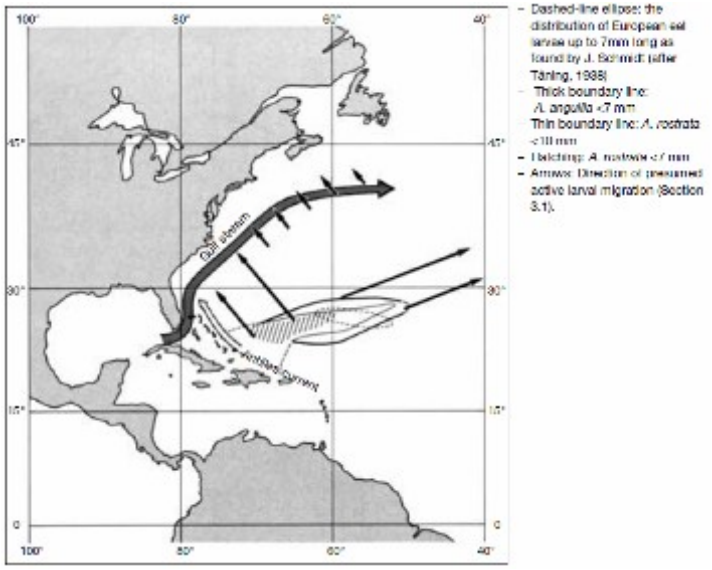
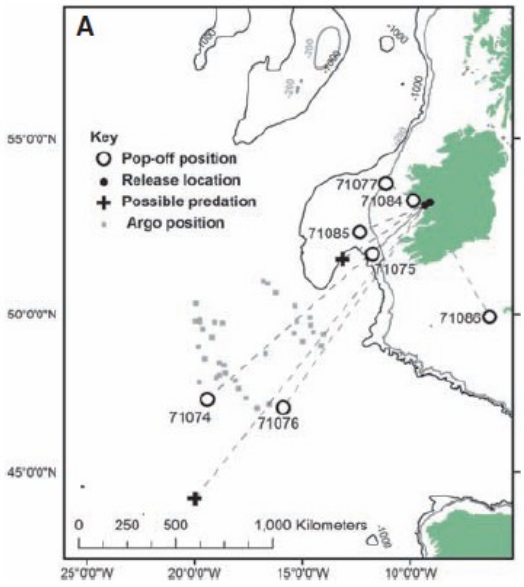


Figure 1.1.8. Oceanic currents used by *A. anguilla* to migrate to Europe (dotted line) and to back to reproduction grounds (dashed line) (American Geographical Society, 1993).



Reproduction grounds and migration routes of Atlantic eels (Schoth and Tesch, 1982).



Detection migrating eels using miniaturized satellite transmitter (Aarestrup et al., 2009).

Figure 1.1.9.

Figure 1.1.10. WaveGliders instrument for fisheries and conservation monitoring (Courtesy of Charles Greene, Cornell University-USA).

B. Reproduction under controlled conditions

Oliviero Mordenti (Bologna University, Cesenatico, Italy) and Dariusz Kucharczyk (Varmia-Mazury University, Olsztyn, Poland) are leading two groups working on artificial reproduction, with preliminary good results (personal communications). I argue that these experiments, dramatically important for the research on eel conservation, should be performed in association to fish evaluation, as discussed in this document.

C. Parasite infestation and barriers to migration

The nematode parasite *A. crassus* is very often detected in many European systems (UK>99%, Gollock et al., 2004; The Netherlands up to 90%; Haenen et al., 2010; Turkey 72÷82%, Genc et al., 2003), but rarely in the coastal lagoon of Comacchio (Dezfuli et al., 2009). Infestation of this organism at large scale, was indicated as a possible factor for the decline of European eel (Feunteun, 2002).

The nematode *A. crassus* was introduced by fish mongers into Europe from Asia (Bruslè, 1994) and rapidly invaded most European water ways. It is located to the swim bladder and it hematophageous diet reduces the oxygen availability and the swimming capacity of the eel. It interferes with the success of the transoceanic migration of spawners, thus an additional possible cause of stock depletion (Feunteun, 2002).

Figure 1.1.11. Swim-bladder infected by *A. crassus*. (left, Ph. J. Simon, Institute of Inland Fisheries Potsdam-Sacrow, Germany) and swim-bladder dissection (right, Palstra et al., 2007).

In fact, the parasite physically damages the swimbladder; its functioning in balancing the migrating silver eels in the ocean at the preferred depth, or switching to other depths, is impaired. Compensation for the loss of the upward force exerted by the swimbladder on the eel costs extra swimming energy for the silver eels. The energy store in the fat reserves of the silver eels is suspected to be insufficient to fulfil these extra needs, finally leading to mortality (ICES, 2004). *A. crassus* is a chronic stress factor for the eel (Haenen et al., 2010), as well as it may cause mortality in wild and farmed eels in association with additional stressors (Kirk, 2003). Regarding barriers to migration, in the coastal lagoon we consider only the man-made sluice-gates, used for water inlet/outlet management.

1.2 Age determination

Age estimation provides information on fish life history that is essential for effective fishery management (Cailliet et al., 2001) and, in particular, age at maturation should be considered in a future revision of the European Regulation n.1100/2007, as an important parameter for EMP implementation. Although age determination is difficult and time-consuming, it is relevant for the stock management in extensive breeding and natural environments. In particular, age determination using calcified structures is the basis for most of fisheries management (ICES, 2009). Otoliths are calcified structures, located in the inner ear of most fish as part of the teleost sensory system, providing sense of equilibrium. Otoliths are calcium carbonate deposits on a protein matrix and grow continuously, thus forming annual increments. Successive layers are deposited throughout the life of the fish, and incorporate chemical elements from the surrounding environment, which is a valuable natural tag of habitat use. These deposits were widely used in fish research for age determination, and also to define population structure, birth sources, and migration patterns of teleost fishes (Vasconcelos et al., 2008; Dierking et al., 2011; Tanner et al., 2011), including eels (Laffaille et al., 2007; Daverat et al., 2011; Schaerlaekens et al., 2011).

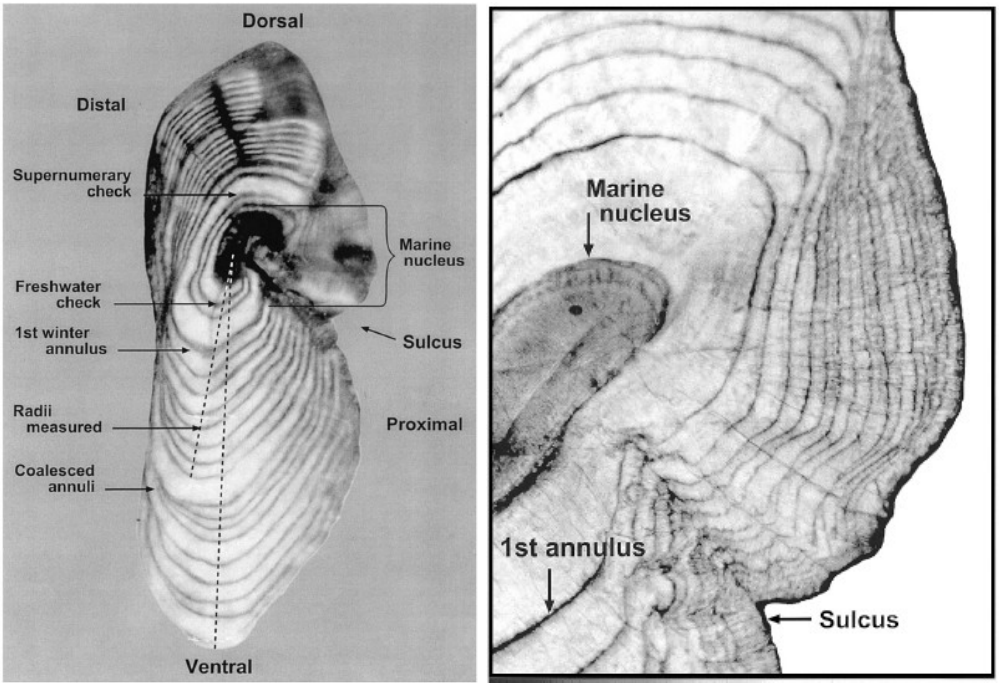


Figure 1.2.1 Otolith description (from Graynoth, 1999).

Otolith reading is considered the most appropriate methodology for age and growth determination, although its accuracy and precision should be improved (Poole et al., 2004). The age determination doesn't include an evaluation of marine nucleus (ICES,

2009), that can have a value from 7 months to 7 years (Lecomte-Finiger, 1992). Furthermore, the first annulus can be influenced by environmental condition and food availability. Age estimation based on otolith readings is routinely used for assessing growth in exploited species, and to improve fishery management (Mercier et al., 2011). However, this approach is destructive in fact it sacrifices fish. Thus, a non-invasive method is preferable. Fish scales can be utilized for determination of fish age; moreover their growth is influenced by their living area, thus providing information on fish life habitats (Jellyman, 1979). They are easy to collect, however the precision of the age estimates based on scale readings was criticized for some species (Schill et al., 2010). Furthermore, annuli may be difficult to distinguish in scales from older fish, leading to age underestimation (Braaten et al., 1999). Sampling area of fish scales can influence age determination (Jellyman, 1979) and the use of fish scales may lead to an aging bias that results from the failure of some fish to form scales during their first stage of life (Hubert et al., 1987).

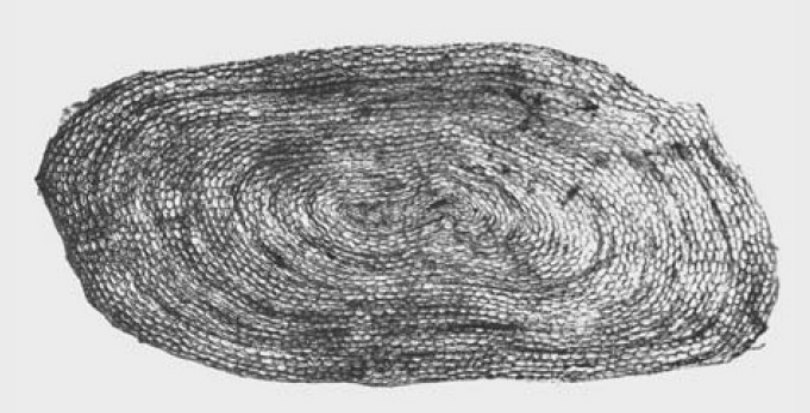


Figure 1.2.2. Photograph of an eel scale (Tesch, 2003).

Eel scales, in particular, are rudimentary and embedded in individual sacs below the epidermis and overlapping is possible, so their collection and use require qualified experts. Tesch (2003) define eels scales usable for age determination only in exceptional cases.

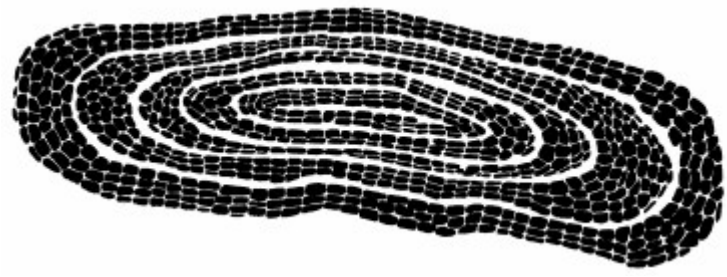


Figure 1.2.3. Eel scales with five age bands; in this drawing, bands are exaggerated (Frost, 1945)

Moreover, although otolith reading provides more precise estimates of age, it is destructive; scales reading is less precise, but is non-destructive, and overall produces good results (Abecasis et al., 2008).

1.3 Effectiveness of Eel Management Plan

In European Union, the Eel Management Plan (EMP) is a mandatory document that local authorities have to define, for monitoring and restoration of eel population. This act is developed on a river basin scale. The EMP is imposed on the EU Members States by the European Council, by the Regulation No 1100/2007. A revision process is forecasted since the middle of 2012.

Because of this Regulation, the 40% of the silver eel biomass should be released into the sea, but no common tools are provided for measurements of biomass and for evaluation of fish health. So, what are the features of fish to release in open waters? But, more in general:

How to:

a. evaluate the fish condition?
b. evaluate the biomass?
c. European Restocking Areas

A. Evaluation of fish condition.

Morphometric parameters, condition indexes and biomarkers can be used to assess the health status of fish, including eels (Roche et al., 2000; Guimaraes et al.,2009).

B. Evaluation of biomass.

For this point, could be useful the non-intrusive method presented by Bilotta et al. (2011). It means of a device (property of the UK-EPA), involving a fixed-position, high frequency multi-beam sonar, that permit a constant surveillance of fish movements. Experimental findings demonstrate the capabilities of this monitoring technique and its usefulness both as a tool to assess the compliance with conservation targets and as a tool to evaluate the success of conservation measures for elusive aquatic species such as *A. anguilla*. Because of its layout, it could be easily applied to coastal lagoons, that often are linked to the sea by marine channels.

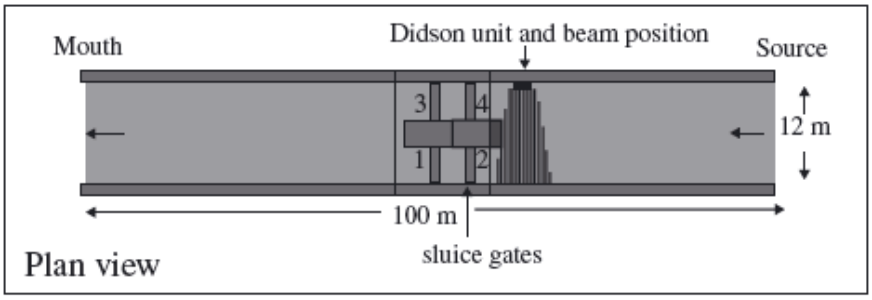


Figure 1.3.1. A device for surveillance of eels movements through a river or a marine channel (Bilotta et al., 2011. The device is property of the UK-EPA).

Furthermore, effectiveness of the EMP is affected by a lack in financial sustainability of monitoring and restoration of stock and a lack of tools for fish evaluation (growth condition and health status).

Article 2 **Establishment of Eel Management Plans** Omissis 4. The objective of each Eel Management Plan shall be to reduce anthropogenic mortalities so as to permit with high probability the escapement to the sea of at least 40 % of the silver eel biomass relative to the best estimate of escapement that would have existed if no anthropogenic influences had impacted the stock. The Eel Management Plan shall be prepared with the purpose of achieving this objective in the long term. Omissis 8. An Eel Management Plan may contain, but is not limited to, the following measures: — reducing commercial fishing activity, — restricting recreational fishing, — restocking measures, — structural measures to make rivers passable and improve river habitats, together with other environmental measures, — transportation of silver eel from inland waters to waters from which they can escape freely to the Sargasso Sea, — combating predators, — temporary switching-off of hydro-electric power turbines, — measures related to aquaculture. Omissis

Table 1.3.1. The statement of article 2 of the Regulation No 1100/2007 of European Commission (EC, 2007).

C. European Restocking Areas

Furthermore, the Eel Management Plan should consider the possibility to design European Restoration Areas, defined as habitat suitable for eel restocking, both for environmental conditions (i.e. water quality, food web, climate, …) and management tools (e.g. inlet/outlet of water in/from closed lagoon managed by sluice gates, …).

1.4 Comacchio lagoon and its eel fishery

This work has been performed on the eel stock of the Comacchio lagoon, a wetland located in Northern Italy, between Venice and Ravenna, on the Adriatic Sea.

The Comacchio lagoon is a brackish coastal lagoon of about 100 km2 (Fig.1) and a maximum water depth of about 1 m. The lagoon is linked to the Adriatic Sea by two marine channels and the inlet of marine water is managed by two sluice gates. Rarely, fresh water is collected by pumps from the Reno River that flows at the southern border of the lagoon.

Depth varies from 0.5 to 1.0 m and the salinity from 20 to 40 psu and at present the total area available for aquaculture is about 80 km2.

In 1970s-‘80s the lagoon hosted an extensive European eel farm (Tesch, 2003) and since 1985, the lagoon suffers for eutrophication due to a persistent bloom of picocyanobacteria, (Sorokin and Zakuskina, 2010) although recently some recovery has taken place (Munari et al., 2003; Munari and Mistri, 2010).

The Comacchio lagoon is characterized by a traditional form of aquaculture, known as “lagoon culture”. In this system, various fish species including anchovies, sea bass, and so on, enter the lagoon in spring, grow during the summer, and are trapped in autumn when they attempt to return to the sea (FAO, 1979). Eels grow for a longer period, and they are captured after up to 10 years. The hatchery provides fishes with a good quality (Melotti et al., 2007).

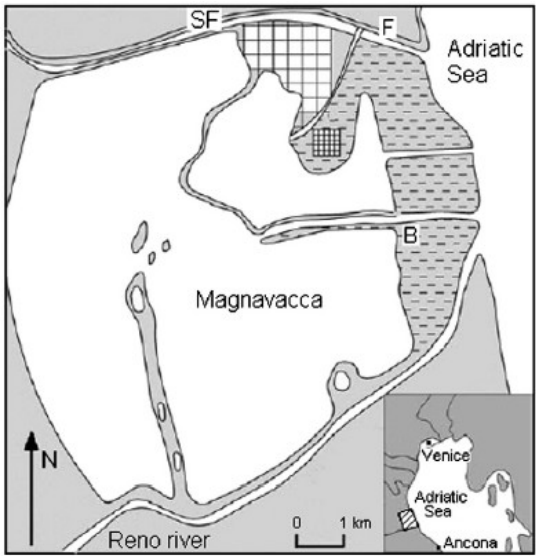


Figure 1.4.1. Map of the Comacchio lagoon (hatchery station Foce - SF , Foce – F and Bellocchio - B channels, from Sorokin, 2010)

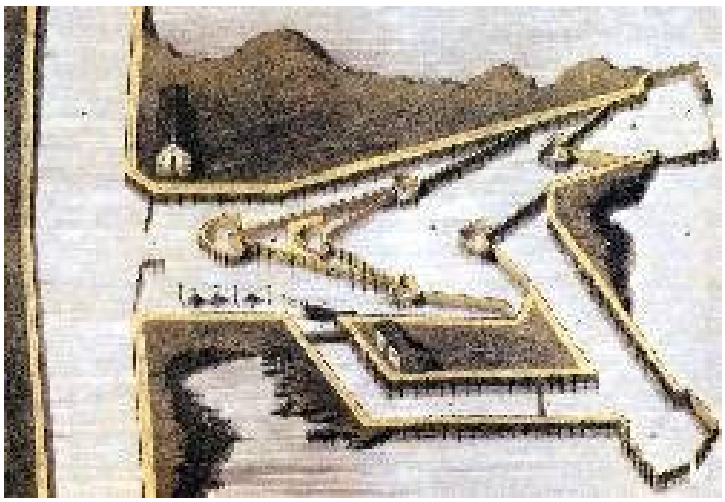


Figure 1.4.2. Drawing of a hatchery station in Comacchio, with the *lavoriero* (Coste, 1865)

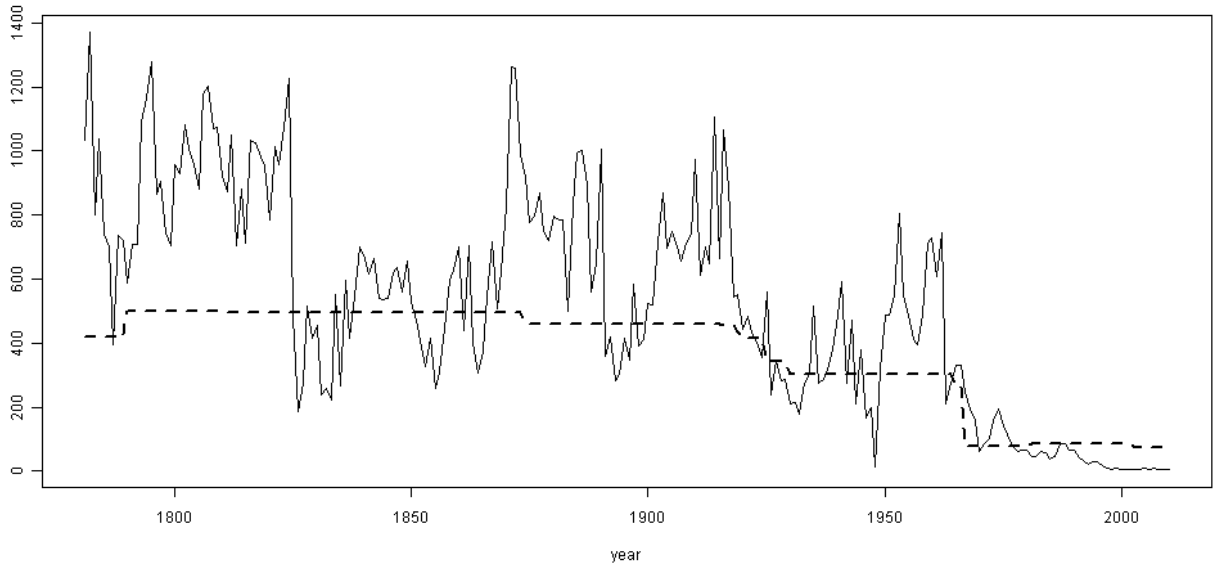


Figure 1.4.3. Capture of eels in the Comacchio lagoon (in tonnes, continue line) and surface of the lagoon (in km2, dashed line), 1791-2010.

The eel fishery in the Comacchio lagoon is performed by the *lavoriero* (Bellini, 1899), a V-shaped fixed traditional fishing weir (Tesch, 2003), although the use of the fykenets is sometimes permitted. Although fykenet eel fishery is the most profitable fishery in coastal lagoons (Psuty-Lipska and Draganik, 2005), the lavoriero is preferred because it captures only migrating fish as they move from the lagoon towards the open sea. Lavoriero therefore is a useful tool for sustainable fishery. Annual records of eel fishery in the Comacchio lagoon are known from 1781 to 2010. These records indicate that eel fishery productivity decreased progressively from 1.40 ton/km^2 in the 1970s, to 0.72 in the 1980s, 0.31 in the 1990s, and 0.08 ton/km^2 in the last decade.

For specimens captured by the lavoriero, Colombo et al. (1984) reported total body length at sexual differentiation was 250-330 mm for males and 300-450 mm for females. Similar differences were reported by Carrieri et al. (1992) who showed a maximum length of 465

mm for males and a minimum length of 525 mm for females at the migrating stage.

Because of the low productivity of the Comacchio lagoon (<0.1 ton/km^2), a low fish density is assumed. This enforces the hypothesis that eels growing at low density tend to differentiate as females (Tesch, 2003; Davey and Jellyman, 2005).

Figure 1.4.4. Capture of eels in the Comacchio lagoon (tonnes), 1980-2010.

As stated in 1.1, the eel population of Comacchio is almost free from one of the main threats of extinction, the nematode parasite *A. crassus,* (Dezfuli et al., 2009).

2. Scope of the thesis

Casone Coccalino, Comacchio lagoon (Ph. A. Kiwan)

The objective of this thesis was to evaluate the condition of eels captured during the reproduction migration from internal waters to open sea. Eels were studied in growth, age, and health status.

The study area was the Comacchio lagoon, a brackish coastal lagoon in Italy, well known as an example of suitable environment for eel fishery, where the capability to use the local natural resources has long been a key factor for a successful fishery management.

For fish evaluation, the SEELF (Sustainable EEL fishery) Index, was developed in two versions: SEELF A, to be used in field operations (catch&release, eel status monitoring) and SEELF B to be used for quality control (food production) and research (eel status monitoring).

SEELF includes morphological parameters and (version B) internal indices and blood parameters. Health status was evaluated on the basis of SEELF Indices, age and biomarker analysis (ChE).

This approach is reliable both for aquaculture and for biodiversity conservation. Particular regard was paid to identify areas where the EMP can be performed effectively. This effort aimed at designing tools for fish evaluation, in order to improve the effectiveness of the Eel Management Plan imposes on the EU Member States by Regulation No1100/2007.

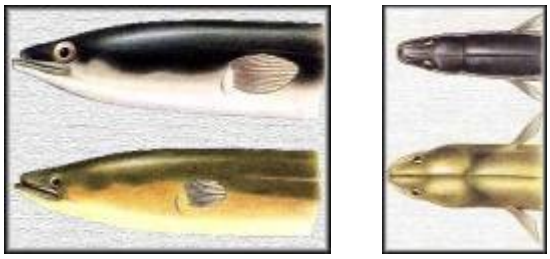
An important conclusion expected from this study was to establish whether the Comacchio lagoon is an appropriated area where an effective EMP can be performed, in agreement with the main features (management of basins, reduction of mortality due to predators,etc.) highlighted for designation of European Restocking Area (ERA).

3. Materials and methods

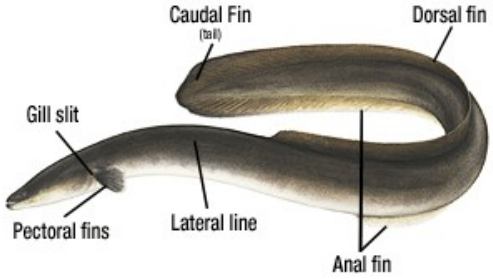
Silver eels captured with *lavoriero* (photo Milko Marchetti).

3.1 Harvest of eels

Silver eels were collected in Autumn, using the *lavoriero* (described in 1.4), in 2009, 2010 and 2011. Two samples were considered: “population” (randomly sampled by captures at lavoriero) and “selected” (sampled using the ASR – Adequate Sampling Ratio). Because of this work aims to describe silver eels migrating from internal water to the sea, the ASR was developed in order to sample female specimens in the fourth stage of silvering (migrating eel), according to Durif et al., 2009.



External features of silver (top) and yellow (bottom) eels
(from www.ittiofauna.org)



Eel external anatomy
(from www.myfalls.com)

Figure 3.1.1.

ASR states that an eel captured in the Comacchio lagoon shows

$$W>L/0.785$$

it has a Silver Index of 4 an, thus, is definable as migrant (W – total weight in grams and L – total length in mm).

So,

$$ASR= L / (W*0,785)$$

and

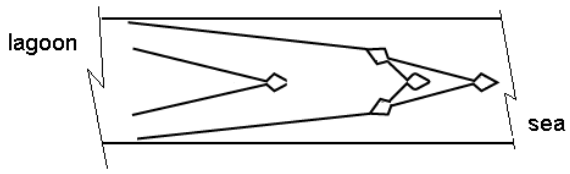
$$\text{migrating eel} \rightarrow ASR<1$$

$$\rightarrow W_{eel} > W_{threshold}$$

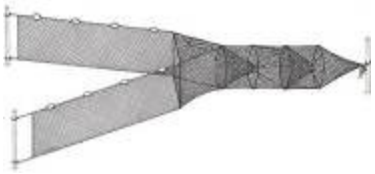
where

W_{eel} is the weight (in g) of the sample
$W_{threshold}$ is the weight (in g) as function of total body length (in mm)

Before of this work, ASR was tested (N=269) with a 100% efficiency. Previous study also shows that fishes harvested by the lavoriero were migrant at 97%, while specimens captured by the fyke-nets were only at 69% migrating (see 3.2.1 for explanation on migrating eels evaluation).



Scheme of the lavoriero



Drawing of fykenets

Figure 3.1.2.

The following table summarized $W_{threshold}$ for sample from 700 to 1000 mm in total body length:

Length	Weight threshold	Length	Weight threshold	Length	Weight threshold
[mm]	[g]	[mm]	[g]	[mm]	[g]
700	900	800	1020	900	1150
710	910	810	1040	910	1160
720	920	820	1050	920	1180
730	930	830	1060	930	1190
740	950	840	1080	940	1200
750	960	850	1090	950	1220
760	970	860	1100	960	1230
770	990	870	1110	970	1240
780	1000	880	1130	980	1250
790	1010	890	1140	990	1270
				1000	1280

Table 3.1.1. Value of total weight vs total length, for selection of silver eels in the Comacchio lagoon.

The following table summarizes the database used in this work:

Year	*Description*	*N*	*Date of sampling*
2009	Randomly sampled	96	19/11/2009
	Selected with ASR	8	10/12/2009
2010	Randomly sampled	100	02/12/2010
	Selected with ASR	8	01/12/2010
2011	Randomly sampled	100	03/11/2011
	Selected with ASR	8	10/11/2011

Table 3.1.2. Database of sampled *A. anguilla* from Comacchio lagoon.

Because of currently there are no stunning methods commercially available that immediately induce unconsciousness in eels (EFSA, 2009), the specimen were fast decapitated after a short (2-3 minutes) storage in ice. On the other hand both eugenol and MS-222 anaesthesia does not cause irreversible damage in Siberian sturgeon (*Acipenser baerii*) caused erythrocyte swelling and haemolysis but no significant changes were noticed in the albumin and glucose concentrations and the activity of lactate dehydrogenase, aspartate aminotrasferase and creatinkinase (Gomulka et al, 2008).

3.2 Eels measurements

3.2.1 Stock sampling

Eels (N=100 per year) randomly sampled by captures at *lavoriero* were classified as "Group A". The specimens were captured by the lavoriero and measured for different parameters:

L total body length (±5 mm)

W total body weight (±10 g)

ED eye diameters (horizontal – EDh, and vertical – EDv, ±0.01 mm)

MD median eye diameter = (vertical eye diameter + horizontal eye diameter)/2

Lf pectoral fin length (±0.01 mm)

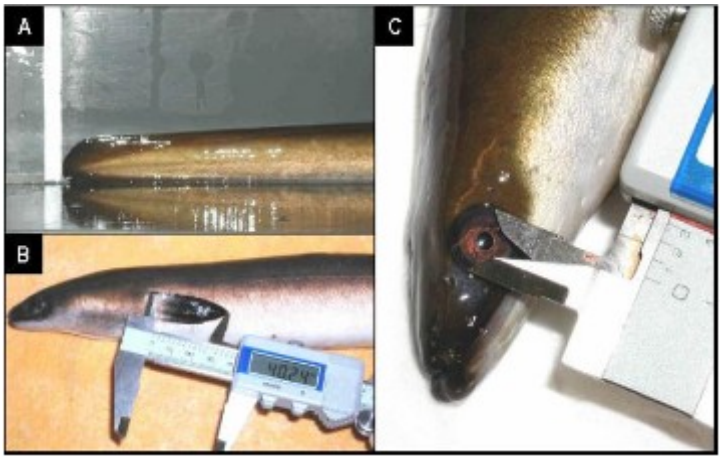


Figure 3.2.1. Estimates on *A. anguilla*, total body length (a), pectoral fin length (b) and horizontal eye diameter (c) (from EELREP, 2005).

The following indices were calculated:

a. Condition factor

$CF = 10^5 \cdot W/L^3$ according to Fulton (1904);

This is the most common morphometric index for evaluation of physical condition, and it is a simple method for monitoring changes in fish health. This was used as a general health indicator (growth, nutritional state and energy content).

b. Eye Index

$EI = 10^2 \cdot ((EDh + EDv)/4)^2 \pi/L$ according to Pankhurst (1982)

When EI > 6.5, the eel is classified as mature. This index doesn't provide a separation between yellow/silver eel and will be used in association with other parameters (e.g. Silver Index and Gonado-Somatic Index) for evaluation of sexual maturity of eels.

c. Silver Index

SI — according to Durif et al. (2009) calculated with L, W, Lf, MD.

Silver Index includes two stages for males (I for residential and MII for migrant) and five for females (I and FII for residential eel, FIII for pre-migrant, FIV and FV for migrant specimens). This index was developed on *circa* 1200 eels, captured in six locations in France with several tools (e.g. elecrofishing, eel pots, fyke nets, weir, stow net).
The Silver Index is based on external body estimates (total body length, body weight, pectoral fin length and mean eye diameter) and the stage of an eel is assigned as the highest of the following sums:

SI = - 61.276 + 0.242 L – 0.108 W + 5.546 MD + 0.614 FL
SFII = - 87.995 + 0.286 L – 0.125 W + 6.627 MD + 0.838 FL
SFIII = -109.014 + 0.280 L – 0.127 W + 9.108 MD + 1.182 FL
SFIV = -113.556 + 0.218 L – 0.103 W + 12.187 MD + 1.230 FL
SFV = -128.204 + 0.242 L – 0.136 W + 12.504 MD + 1.821 FL
SMII = - 84.672 + 0.176 L – 0.116 W + 12.218 MD + 1.295 FL

For example, if the maximum value is provided by SFIV, the eel is classified as FIV, i.e. female at the fourth stage of silvering.

For Durif et al (2009), the precision of Silver Index (defined as correctly stage identification), varies from 70% (FIII), 91% (FIV, FV and MII), up to 95% (FI and FII). A mean precision of 92% was calculated.

Before to use this index on the stock of the Comacchio lagoon, a comparison between external evaluation (colour, eye's index, condition factor) and calculated Silver Index was performed (N=367). SI was evaluated as adequate for silvering stage identification. Because of the Silver Index provides is an approximation, during this work it was used in association to other parameters, in order to the select mature fish appropriately: total length (historical data are available for the study area) and gonado-somatic index (see point "e" of this paragraph). These parameters were compared with SI also in EELREP (2005), see the following table.

	Stage	Description
Resident	I (mean length of 40 cm)	Gonads are hardly developed: Testes are not visible, ovaries appear as translucent strips. GSI<0.5%
	FII (mean length of 53 cm)	Ovaries are visible and more opaque. Mean GSI=0.5%
Pre-migrant	FIII (mean length >50 cm)	High levels of growth hormone, and beginning of gonadotropin synthesis Mean GSI=0.8%
Migrant	FIV (mean length >50 cm)	Cessation of feeding, first downstream movements Mean GSI=1.5%
	FV (mean length >50 cm)	Actively migrating eel Mean GSI=1.7%
Migrant	MII (mean length=39 cm)	Visible testes although they are hardly developed. Cessation of feeding and migratory movements. Mean GSI=0.16%

Figure 3.2.2. Relationship between stage of silvering and body estimates, gonads evaluation and other observations on behaviour and anatomy of eels (EELREP, 2005).

Historical data for Silver Index were calculated also on eels captured from 2006 to 2008 in the Comacchio lagoon and other coastal lagoons of North-Western Adriatic Sea coast (N=502).

3.2.2 Silver eels sampling

A second group of eels (N=8 per year) was collected simultaneously to Group A and classified as Group B. Fish were selected using the Adequate Sampling Ratio (described in 3.1), with ASR<1. These fishes were measured for the same parameters listed for Group A. Further parameters and indexes were also evaluated:

d. Hepato-somatic index

HSI = (WL/W)*100

where WL is the liver weight; WL and W in the same units;

Since the liver is the major detoxification and lipid storage reserve organ, changes in weight of this organ will relate to detoxification and energy storage (Maes et al., 2005). Furthermore, for species that store energy reserves hepatically, the hepatic somatic index is also useful in ecotoxicological investigations (Ribeiro et al., 2005).

e. Gonado-somatic index

GSI = (WG/W)*100

where WG is the gonadal weight; WG and W in the same units;

Gonad differentiation and maturation are more related to attained body size and environmental conditions, rather than age (Grandi et al., 2003). Gonads weight is a obvious indicator of the sexual maturation process (Tesch, 2003; EELREP, 2005) and it is easily applicable on samples sacrificed for age determination by otolith readings (see 3.3.2).

f. Hematocrit

Hematocrit (HT), i.e. packed blood red cell volume (PCV), was determined using capillary tubes, after centrifugation at 1600xg for 30' at 4°C. In fact, when heparinized blood is centrifuged, the red blood cells become packed at the bottom of the tube, while the plasma is left at the top. The ratio of the volume of packed red cells to the total blood volume is readable on a scale, as percentage.

This parameter was evaluated on eel blood collected into heparinized tubes immediately after fast decapitation of the individuals.

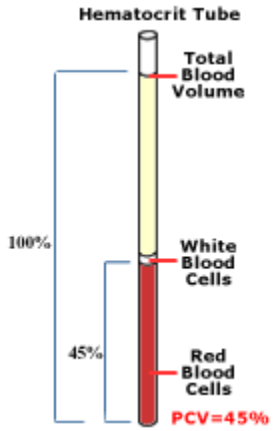


Figure 3.2.3. Estimate of PCV using an Hematocrit Tube.

g. Blood cells counts

Red blood cell (RBC) and white blood cell (WBC) counts were calculated using a Neubauer hemocytometer and Natt&Herrick's solution (Natt and Herrick, 1952), in 1:200 and 1:50, respectively, dilution (Rossi, 2009).

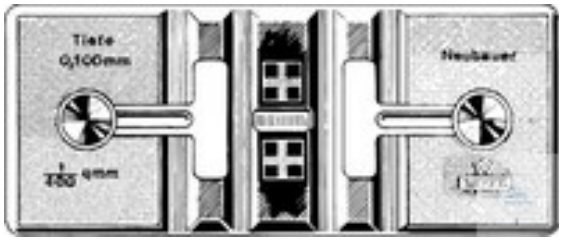


Figure 3.2.4. Drawing of the Neubauer hemocytometer (www.wenk-labtec.de).

These parameters were evaluated on eel blood collected into heparinized tubes immediately after fast decapitation of the individuals.

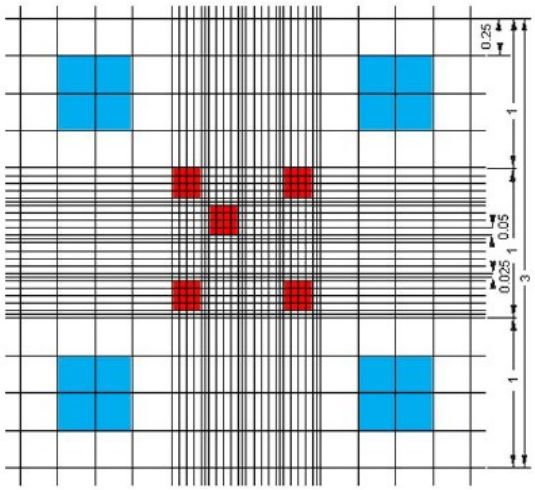


Figure 3.2.5. Scheme of Neubauer hemocytometer's chamber for estimates of RBC (red squares) and WBC (blue squares). Measurements in mm. Scale 2:1. (Rossi, 2009)

h. Presence of the nematode *Anguillicola crassus*, by dissection of the swim-bladder; if present, *A. crassus* is easily detectable because specimens are located in a empty sack as the swim-bladder is.

i. Age determination. See 3.4.

3.3 SEELF Index

The Sustainable EEL Fishery (SEELF) Index was designed for the evaluation of conditions of migrating fish. Two versions are proposed:

"SEELF A" to be used in the field for rapid evaluation of fish condition, without sacrificing the animals; and,

"SEELF B" to be used for research and quality control purposes.

SEELF A consists of four objective parameters including (1) ASR or the Adequate Sampling Ratio; (2) Silver Index; (3) Eye Index; and, (4) Condition Factor. SEELF A is based only on external measurements and it is therefore suitable to be used in the field.

SEELF B consists of the four indices noted for SEELF A and four additional parameters, including an internal index (hepato-somatic Index, HSI), two haematological parameters (RBC and HT) and a subjective evaluation of skin (SE, skin evaluation). For SEELF B determination, fish are sacrificed by decapitation, blood samples taken for haematocrit (HT) and red blood cell counts (RBC), and the liver weighed for calculation of HSI. Skin evaluation (SE) is performed without any manipulation or damage to the carcass. Parameters are evaluated on the basis of a threshold or a range (see 3.3.2). To assign a numerical value to variables, each parameter that represents a normal condition is replaced by a one; otherwise by a zero. As only exception, SE is classified on a scale of 0 to 1 (0=severe damage, 0.5=moderate damage, 1=no damage).

The sum of all values is normalized to 10 and assigned as value of SEELF. This ranking system assures that every sample is evaluated from 0 (min) to 10 (max); therefore the SEELF index is always a number in the range 0÷10.

3.3.1 SEELF approach

The SEELF Index aims to quickly evaluate a fish, both for fishery management (catch&release, capture for food processing,…) and biodiversity conservation (health evaluation, release to open water,…). SEELF provides an easy and raw methodology, usable by fishermen, conservationists, researchers and protected area managers. When the target is the evaluation of a trend, the ranking system permits the comparison of fish sampled in different times (e.g. the set of parameters can change) or in different habitat (e.g. the parameter's threshold/range can change). Thus, SEELF is a tool for evaluation and comparison, useful in stock management.

3.3.2 SEELF calculation

The following table summarizes the parameters included in the SEELF Index:

N	*Parameter*	*SEELF A*	*SEELF B*
1	Adequate Sampling Ratio	✓	✓
2	Condition Factor	✓	✓
3	Eye Index	✓	✓
4	Silver Index	✓	✓
5	Hepato-somatic Index	✗	✓
6	Hematocrit	✗	✓
7	Red blood cell count	✗	✓
8	Skin Evaluation	✗	✓

Table 3.3.1. List of parameters included in the two version of the SEELF Index.

Although several scenarios will be investigated (see chapter 4), a description of the parameters and references for normal conditions are provided:

1. The Adequate Sampling Ratio was properly designed for the capture of migrating eels, and ASR is imposed to be < 1.
2. The Condition Factor was widely used by several author as a general health indicator; values of 0.17 or less were estimated for eels growth in polluted environment (van der Oost et al., 1996; C. Gravato et al., 2010) while 0.18 or greater were estimated for healthy fishes (Maes et al., 2008; Palstra et al., 2008; Palstra et al., 2010). Maes et al. (2005) show a decrease of CF with the increment of pollution detected in the water. A value of >0.20 is here considered as normal condition.
3. The Eye Index defines mature eels with EI ≥ 6.50 (Pankhurst, 1982), as used recently by van Ginnecken et al. (2007) and Valbonesi et al. (2011) and confirmed by Palstra et al. (2008) and Palstra et al. (2010).
4. The Silver Index classify mature female eels in the stage FIV and FV (Durif, 2009) and this statement was proved on the stock of the Comacchio lagoon (Valbonesi et al. 2011). In order to identify migrating eels, SI ≥ 4.
5. The Hepato-somatic Index for healthy eels is variable from 1.00 to 1.50, as confirmed by several authors (EELREP, 2005; Ribeiro et al., 2005; Palstra et al., 2010; Valbonesi et al. 2011).
6. Values of Hematocrit are rarely present in literature, as well as Ghittino (1983) reports 44%, van Ginneken et al. (2009) 37.7%, Caruso et al. (2010) from 28.00 and 40.20% and Palstra et al. (2010) from 24.35 to 39.60%. Thus, a normal range for this parameter is here assumed as 35%<HT<45%.

7. Red blood cell count values are also very rarely present in literature; Ghittino (1983) reports a density of $1.44 \cdot 10^6$ erythrocytes/mm^3 (eels from aquaculture), while Rossi (2009) reports $1.88 \cdot 10^6$ erythrocytes/mm^3 (eels from the same habitat).

Thus, a normal range for this parameter is here defined as $1.40 \cdot 10^6 < RBC < 2.2 \cdot 10^6$ erythrocytes/mm^3.

8. The Skin Evaluation ranking was designed as a qualitative evaluation of external features of the fish:

SE	*Description*	*Note*
0	Macroscopic evidences of parasite infections or other pathologies and/or severe damages to the fish carcass.	*Study on parasite infestations in the geographical area, can support the investigations on samples.* *Light damages on the carcass may be produced by capture tools (e.g. fyke-net).*
0.5	No macroscopic evidences of parasite infections or other pathologies and/or moderate damages to the fish carcass.	
1	No macroscopic evidences of parasite infections or other pathologies and absence or very light damages to the fish carcass.	

Table 3.3.2. Description of the parameter "Skin Evaluation" for the calculation of SEELF Index

An example of macroscopic evidence of disease is the bloody inflammatory red lateral line, as reported by Van Ginnecken et al (2009) in eels during swimming experiments or the cauliflower-like growths disease (Tesch, 2003).

SEELF index is calculated as:

$$\frac{\sum_{i=1}^{N} P_i}{N} * 10$$

where Pi is the value of the i-th parameters and N=4 (SEELF A) or 8 (SEELF B).

The following table summarizes the values assigned for calculation of the SEELF Index.

	Range		*Threshold*	*Pi=1 if the value*
Parameter	*Minimum*	*Maximum*		*measured is*
Condition Factor			0.20	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<
Skin Evaluation			0.50	>
Liver-somatic Index	1.00	1.50		Included
HT	35	45		Included
RBC	1,500,000	2,200,000		Included

Table 3.3.3. Values of ranges and thresholds assigned to SEELF's parameters. Units are omitted.

3.4 Age determination

3.4.1 A new low-cost device for otolithometry

Otolithometry is the age determination by reading of alternate dark (winter) and white (summer) bands of the otolith. After length estimate, otolith is encapsulated, cut along the transverse axis and stained. For an appropriated reading, otolith have to be polished to the centre, in order to read the marine nucleus and all annuli (Panfili and Ximenes,1992). Usually this operation is manual, through a time-consuming procedure that lacks in precision. A new low-cost device (otolith polisher, OP) was used in this work. OP consists of a low power electric engine, which holds a rotating disc (Ø=130mm) with an sandpaper (1000 grade) on the surface, and of a spindle located on a mobile carriage. The progress of the carriage hosting the spindle toward the rotating disc is measured by an analog comparator (± 1/100 mm). The otolith, embedded as described in 3.4.2, is placed into the spindle and the carriage moves slowly and steady till to polish the otolith up to its middle.

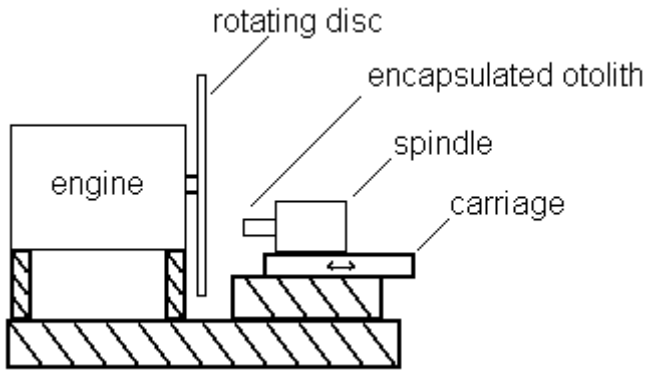


Figure 3.4.1. Scheme of Otolith Polisher, a new low-cost device developed for eel's otolith reading (Brunelli).

As discussed by Panfili and Ximenes (1992), an incorrect preparation of otolith brings to a false interpretation of the visible section plan after the grinding, as explained by the image above.

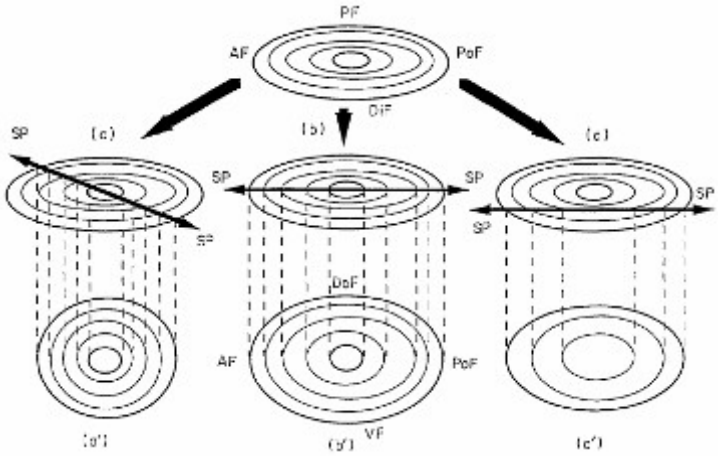


Figure 3.4.2. Errors due to imprecise otolith reading (Panfili and Ximenes, 1992. AF, anterior face; DiF, distal face; DoF, dorsal face; PF, proximal face; PoF, posterior face; SP, sectioning plane; VF, ventral face).

A careful otolith preparation and use of OP reduce mismatching in otolith readings, improving the effectiveness of the technique.

3.4.2 Otolith reading

Sagittal otoliths were sampled, washed with double-distilled water and measured in length, encapsulated in resin (GC UNIFAST general purpose acrylic resin, GC Europe, Leuven, Belgium) and polished with abrasive paper (1000 grit), using the device described in 2.3.1, until the marine nucleus was visible. The polished otolith was treated with 1% HCl for 60 seconds and stained with Neutral Red 3.3 g/L for 30 seconds (ICES, 2009). Stained otolith was examined microscopically (20-fold magnification), using reflected light; marine nucleus, fresh water check, first annulus and other annuli were identificated.

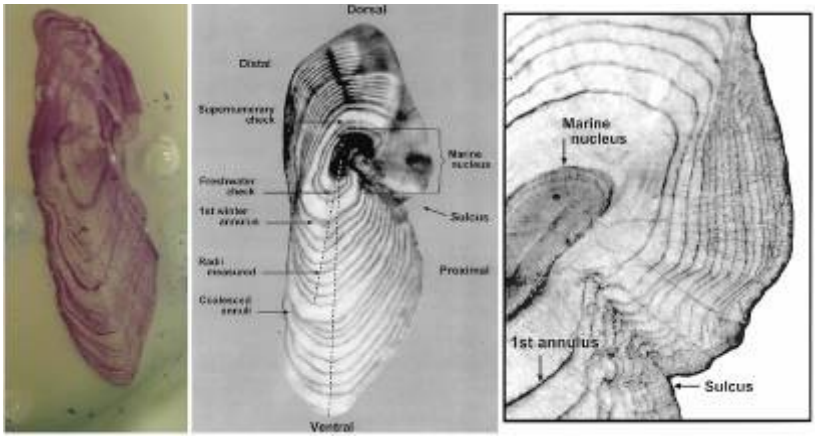


Figure 3.4.3. A 2010 stained otolith (left) and explanation of otolith reading (right, black/white, burned otolith of Australian eel, Graynoth, 1999).

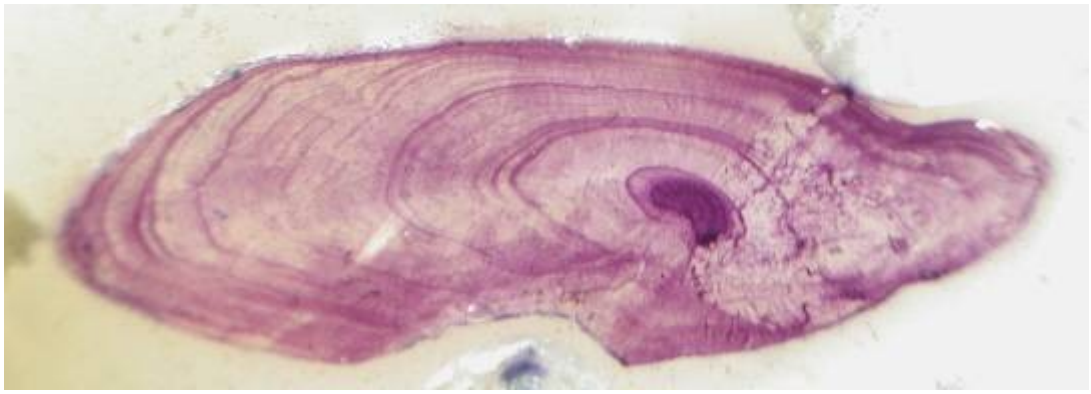


Figure 3.4.4. A 2010 stained otolith (view at optical microscope, 10x magnifold)

The age was obtained counting the numbers of alternating dark annuli (according to Graynoth, 1999) and assuming a value of 2 years (Lecomte-Finiger, 1992) for the marine nucleus. Otoliths readings were carried out by three individuals independently. When appropriated, otoliths were stored dry at room temperature (RT) in Eppendorf tubes.

3.4.3 Fish scale reading

Scales (N=3 for each specimen) were removed, from the area adjacent to the lateral line, located at two-third of the distance from the anus and the tip of the tail (Jellyman, 1979). These were washed with bidistilled water, placed on a glass slide and examined at the microscope (10-fold magnification). The number of dense bands (see 1.2) was counted. The age was calculated by three experts, independently. Experts had knowledge about size and sex of samples, but had no details on age determination with otoliths which was carried out in parallel (see 3.4.2). When appropriated, fish scales were stored dry at RT embedded between a glass slide and a coverslip.

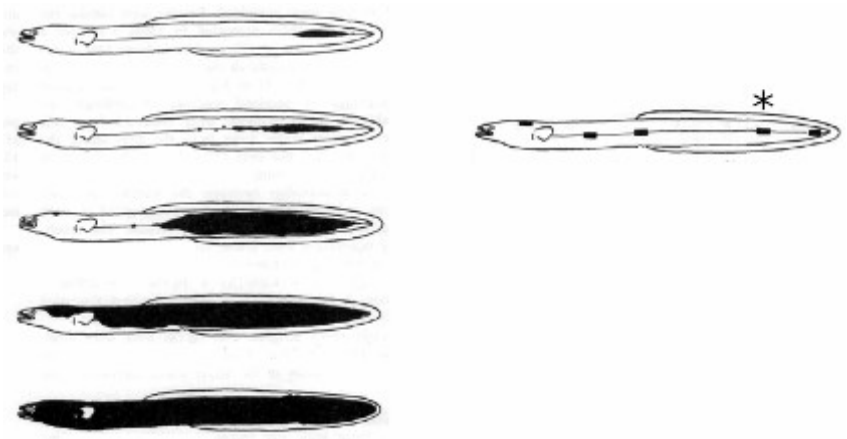


Figure 3.4.5. Successive stages in scale spreading in *Anguilla dieffenbachii* (left) and sampling areas (dashes, right), from Jellyman, 1979. The used sampling area is labelled with *.

3.5 ChE assays

3.5.1 ChE forms

Acetylcholinesterase (AChE; EC 3.1.1.7) and the pseudocholinesterases butyryl-cholinesterase (BChE) and propionylcholinesterase (PChE) (EC 3.1.1.8) activities were determined in eel brain, skeletal muscle and liver homogenized, as described in 3.5.2. The substrate acetylthiocholine iodide (ASCh) was used at 10 concentrations ranging from 0.01 mM to 10 mM in order to evaluate enzyme Km and Vmax values. When different substrates (ASCh, propionylthiocholine, PSCh, or butyrilthiocholine, BSCh) were used, the incubation was carried out as stated above in the presence the 0.5 mM concentration, selected as in the range of the Km values provided for ASCh hydrolysis in preliminary experiments. Concentrations 10-times higher and 10-times lower were also applied.

The sensitivity of eel ChE to inhibition by eserine, BW 284c51, Iso- OMPA and pesticides was assessed after 30 min of pre-incubation at 25°C with the compounds. The substrate was used at 0.5 mM concentration. Stock solutions of eserine, iso-OMPA and organophosphates were prepared in ethanol. Stock solutions of carbamates were prepared in acetone. Effects of the solvents on the enzyme activities were accurately assessed and found to be negligible even at the highest tested concentrations (0.1%). BW284c51 stock solution and diluted solutions for each inhibitor were prepared in ultra-pure water. The enzymatic reaction rate was quantified spectrophotometrically at 405 nm by using a Multi Sample DU800 Beckman spectrophotometer. The incubation was monitored at 1 min intervals for 10 min, and the enzyme activity expressed as $nmol \cdot min^{-1} \cdot mg$ of $protein^{-1}$. In each experiment a blank without substrate was measured to evaluate the reaction of aspecific thiols with DTNB, and a second blank without sample was used to estimate the rate of spontaneous substrate hydrolysis. Data are given as the mean±SE (standard error) of up to 12 individuals assessed independently. For each tissue of each animal, at least 3 separate replicates were carried out.

Data were analyzed fitting experimental curves using the Michaelis–Menten equation, in order to determine the kinetic parameters of ChE, Vmax and Km. The sigmoidal inhibition curves were fitted using the five parameter sigmoid equation from the library of functions of the fitting program used; IC50 values were obtained interpolating the equation using the fitting parameters. Curve fitting was performed using a commercial graphical package (SigmaPlot ver. 9.00, SPSS Science, Chicago IL, USA). One way analysis of variance using Sigma Stat (SPSS Science, Chicago IL, USA) was applied for statistical analysis of the data. Differences were considered significant at $p<0.05$.

3.5.2 AChE

Silver eels were captured as Group B (see. 3.2.2), dry transported and stored 24h in an aquarium containing water sampled from the lagoon. For the assays, the animals were collected from the aquarium, briefly stored on ice, killed through fast decapitation and drained of blood; 2 ml of blood from each individual was collected into heparinized tubes, and immediately centrifuged for 15 min at 2200×g to obtain the plasma fraction, which was separated and frozen at −20°C. The brain, liver and pieces of white skeletal muscle were immediately dissected out, washed with cold physiological saline solution, and frozen in the liquid nitrogen. Samples were kept at −80°C until following analysis.

Acetylcholinesterase (AChE; EC 3.1.1.7) activities were determined in eel brain, skeletal muscle and liver homogenized in ice-cold 100 mM phosphate buffer, pH 7.4 (1/4 W/V) with a tissue grinder. The homogenate was centrifuged at 9000×g (4°C, 30 min) and the clear supernatant was used for the enzyme assays. Enzyme activities were also determined in eel plasma. The sample protein concentrations were estimated according to Lowry et al. (1951), using bovine serum albumin as standard. Enzyme activities were assessed following the method of Ellman et al. (1961). In a typical assay, 0.06 mg of protein from the above described preparations were incubated at 25°C in a final volume of 1.2 ml containing: 100 mM phosphate buffer, pH 7.4 and 0.33 mM DTNB. In plasma samples assays were performed with 0.6 mg of protein.

3.6 Blood biochemistry

Blood samples were collected after fast decapitation, in heparinized tubes. Each blood sample was stored on ice for 30' and then centrifuged for 20 min at 3000xg in a refrigerate centrifuge (4°C) for obtaining the plasma. Samples were analysed using commercial kits (Olympus AU400) with an automated biochemical analyzer (Olympus AU400). A standard biochemical profile was chosen, including: glucose, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), gamma glutamyl transferase (GGT), total proteins, albumin, Ca, P, Mg, Na, Cl and K.
Sampling was performed by the author, while the experimental assays were performed by Prof. Gloria Isani and MSc Barbara Rossi (Department of Veterinary of the University of Bologna) and Dr Nadia Mucci (ISPRA – Institute for Environmental Protection and Research, department of Ozzano Emilia, Bologna).

3.7 Genetic investigation

Portion of pectoral fin was removed from carcass and stored in Ethanol 70% solution in Eppendorf tubes. DNA were extracted using a commercial kit (ZymoResearch Genomic DNA™ Tissue MiniPrep D3050 kit). Filogenesys of mitochondrial aplotype were performed with MEGA 4.0 software, including mtDNA sequences of *A. rostrata* and *A. japonica* (GenBank). The analysis was integrated with Network 4.5.1.6 software.
Sampling was performed by the author, while the experimental assays were performed by Prof. Gloria Isani and MSc Barbara Rossi (Department of Veterinary of the University of Bologna) and Dr Nadia Mucci (ISPRA – Institute for Environmental Protection and Research, Department of Ozzano Emilia, Bologna).

4. Results

Eels loaded in a *bolaga* (buoy), where they are stored before the food processing (photo Milko Marchetti).

4.1 Estimates on eels – Group A

The following table (4.1.1) summarizes the estimates on eels randomly sampled, and defined as Group A, from 2009 to 2011. All measurements are shown in Annex 1. Historical data from Comacchio lagoon (provided by the author), from 2006 and 2008, are also listed. Data from other coastal lagoons are reported in Table 4.1.2, for comparison.

	Units	2006	2007	2008	2009	2010	2011	Mean (2009÷2011)
N		70	99	100	96	100	100	296
L	[mm]	832±4.5	799±5.6	826±4.2	810±8.6	846±5.0	772±5.6	810±4.1
W	[g]	1288±15.4	1155±23.8	1286±22.0	1198±33.7	1404±27.0	1023±23.4	1208±18.6
CF		0.224±0.003	0.224±0.002	0.227±0.002	0.217±0.002	0.235±0.004	0.219±0.002	0.224±0.002
EI		7.93±0.15	10.72±0.16	6.79±0.12	8.13±0.18	10.38±0.13	9.10±0.14	9.22±0.10
SI		3.9±0.03	4±0.03	3.7±0.05	3.8±0.01	4.0±0.02	3.8±0.05	3.8±0.03

Tab. 4.1.1. Group A - morphological estimates and body indices of European eels (*A. anguilla*) caught in the Comacchio lagoon between 2006 and 2011. Data are reported as mean±SE.

Group A morphological estimates and body indices grouped by year of fishery (total number = 565) are summarized in Table 4.1.1. Specimens captured were mature female eels (99.0% in average; 96.9% in 2009, 100.0% in 2010 and 2011), as evaluated on the basis of the body length (minimum length > 600mm), with Eye Index >6.5 in 83.3% in 2009, 100.0% in 2010 and 97.0% in 2011 and SI≥4 in 73%, 98% and 72% in 2009, 2010 and 2011 respectively. Sample with both EI>6.5 and SI<4 were 10.6% in 2009 and 0% in 2010 and 1& in 2011. The 2010 sample show the highest averages in all measurements, while 2009 shows the lowest L (400mm), W (90g), CF (0.13) and EI (3.06) and higher L (990mm) while 2010 report the higher W (2490g), CF (0.49) and EI (13.36).

	Units	P1	P2	VB	P3	P3	FB
		Nov. 2006	Nov. 2006	Dec. 2008	Dec. 2008	March 2009	March 2009
N		14	15	42	43	92	27
L	[mm]	595±15.0	545±5.9	412±3.2	627±9.2	611±6.1	526±11.6
W	[g]	390±27.3	326±12.0	117±2.7	459±30.4	391±15.0	252±16.9
CF		0.183±0.007	0.200±0.004	0.167±0.002	0.178±0.003	0.165±0.003	0.166±0.005
EI		6.89±0.40	6.99±0.35	8.70±0.27	8.73±0.52	6.61±0.19	4.52±0.34
SI		3.1±0.22	3.3±0.23	3.9±0.24	4.2±0.15	3.5±0.11	2.0±0.13
S.B		2.9±0.69	3.3±0.58	4.1±0.22	4.1±0.35	2.0±0.26	0.5±0.19

Tab. 4.1.2. Morphological estimates and body indices of European eels caught in other coastal lagoons between 2006 and 2009. Labels are used to identify private (P1, P2, P3) and public (VB, FB) lagoons. Data are reported as mean±SE.

Data elaborated with R Development Core Team (2011).
The frequency distributions for Group A are shown in graphs.

Fig. 4.1.1. Total body length in mm.

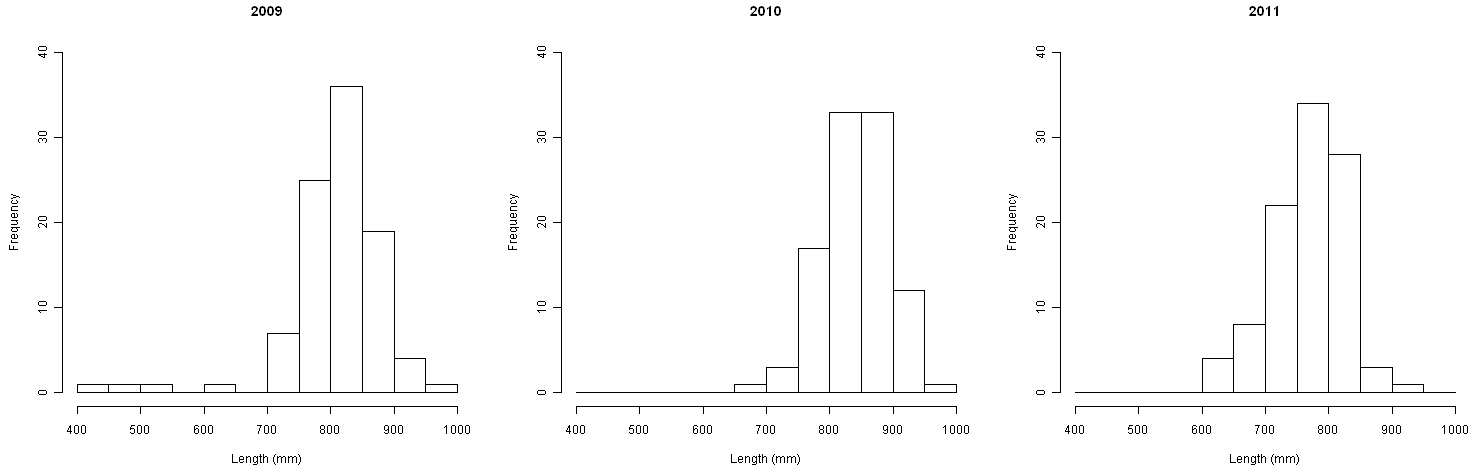


Fig. 4.1.2. Total body weight in g.

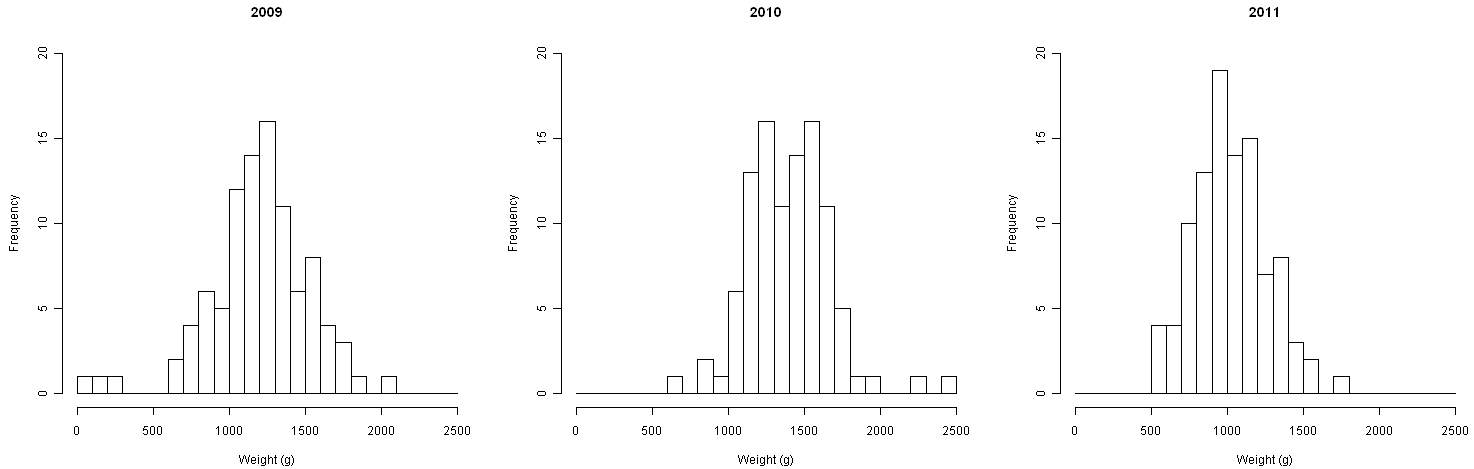


Fig. 4.1.3. Condition Factor

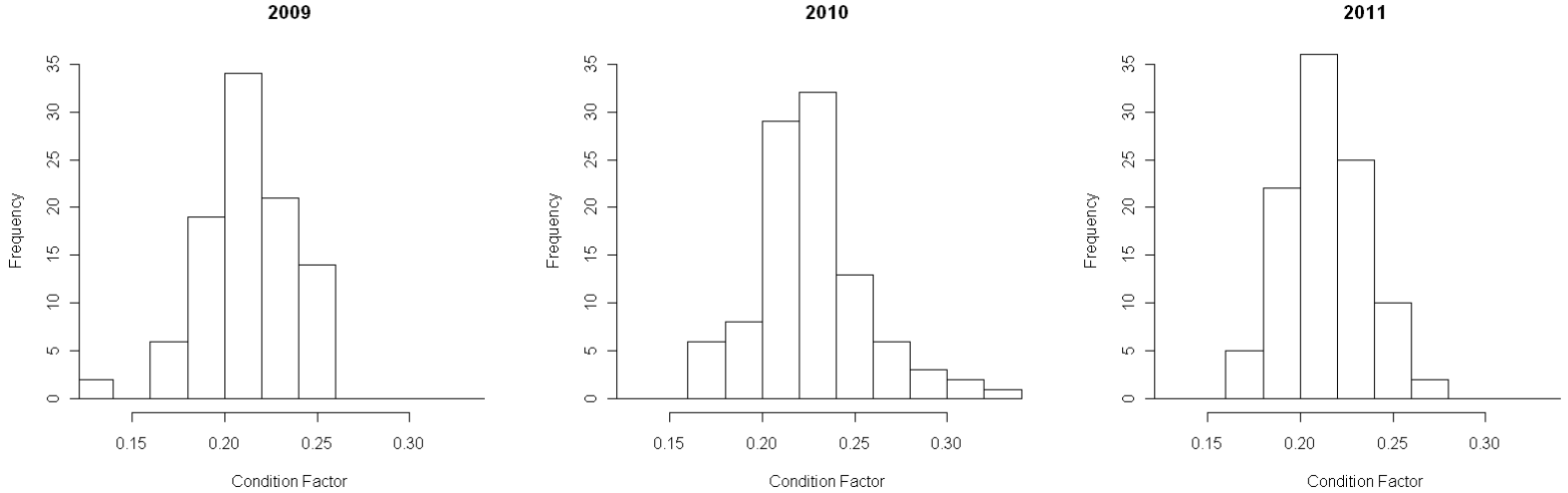


Fig. 4.1.4. Eye Index

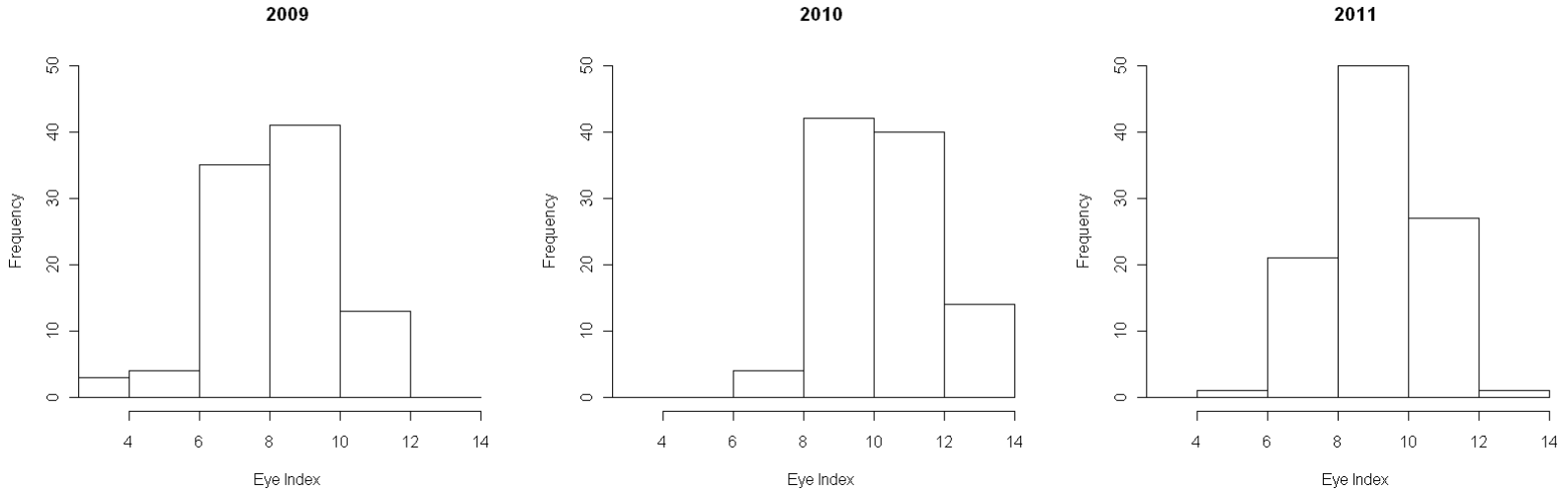
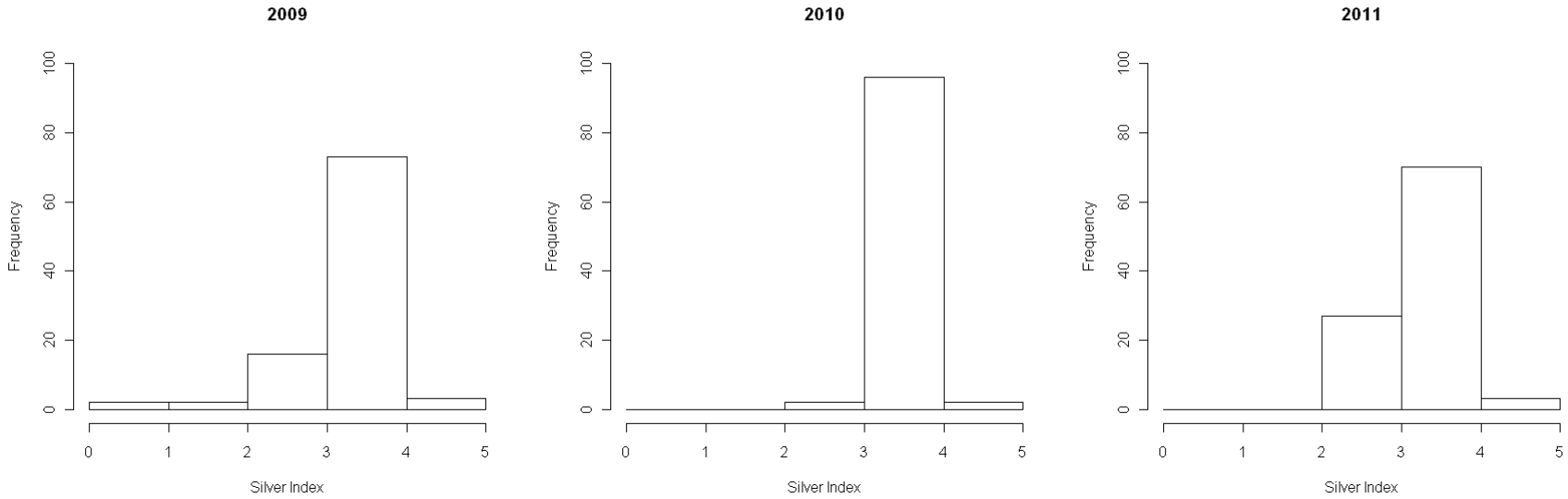


Fig. 4.1.5. Silver Index



4.2 Estimates on eels – Group B

The following table summarizes the estimates on eels selected using the ASR, and defined as Group B, from 2009 to 2011. Nematode *Anguillicola crassus* was never detected. All measurements are shown in Annex 1.

	Units	*2009*	*2010*	*2011*	*Mean*
N		8	8	8	24
L	[mm]	859±8.4	827±20.9	844±12.1	844±8.3
W	[g]	1300±45.2	1300±41.8	1487±60.2	1358±34.7
ASR		0.86±0.02	0.80±0.03	0.73±0.02	0.79±0.02
CF		0.21±0.01	0.23±0.01	0.23±0.00	0.23±0.01
EI		9.04±0.35	9.82±0.16	10.35±0. 43	9.79±0.20
SI		4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00
HSI	[%]	1.22±0.01	1.00±0.01	NA	1.11±0.04
GSI	[%]	1.67±0.07	1.48±0.05	1.82±0.11	1.67±0.06
HT	[%]	36.8±0.5	36.1±1.0	29.3±1.1	33.4±1.1
RBC	[$\cdot 10^6$ /mm^3]	1.78±0.26	1.64±0.08	1.64±0.08	1.69±0.08
WBC	[$\cdot 10^3$ /mm^3]	68.8±8.3	44.6±2.8	65.2±6.1	59.5±4.2
Age $_{\text{otolith}}$	[year]	9.2±0.5	9.0±0.3	8.4±0.3	8.8±0.2
Age $_{\text{fish scale}}$	[year]	9.2±0.5	8.6±0.3	8.8±0.3	6.8±0.2

Tab. 4.2.1. Group B - morphological estimates, body indices, blood parameters and age of European eels caught in the Comacchio lagoon between 2009 and 2011. Data reported as mean±SE. (NA=not available)

Data for HSI in 2011 are not available, because the samples were used for investigation on glucose metabolism in liver, as a key factor for evaluation on success of reproductive migration (Kiwan, 2012). Because of this study on metabolism, the eels sampled in 2011 were selected particularly large in size, in order to have the most mature eels.

Specimens were all mature (EI>6.5, minimum value=7.5) females (L>770mm), with homogeneous Condition Factor and age. 2009 has the lowest Eye Index and the higher value of total Length and WBC. 2010 show the smallest total Length and WBC and a GSI near to 1.50, defined as the reference for female eels at 4th stage of silvering (EELREP, 2005). The 2011 sample has a higher average weight and the smaller HT. 2009 and 2010 report a huge GSI, defining more mature specimens, near (2009) and much over (2011) the reference value for female eels at 5th stage of silvering (EELREP, 2005). Details on age determination are reported in 4.4. The parasite *A. crassus* was not detected.

Data elaborated with R Development Core Team (2011).
The graphs above show trend for each parameter (SI excluded).

Fig. 4.2.1. Total body length in mm.

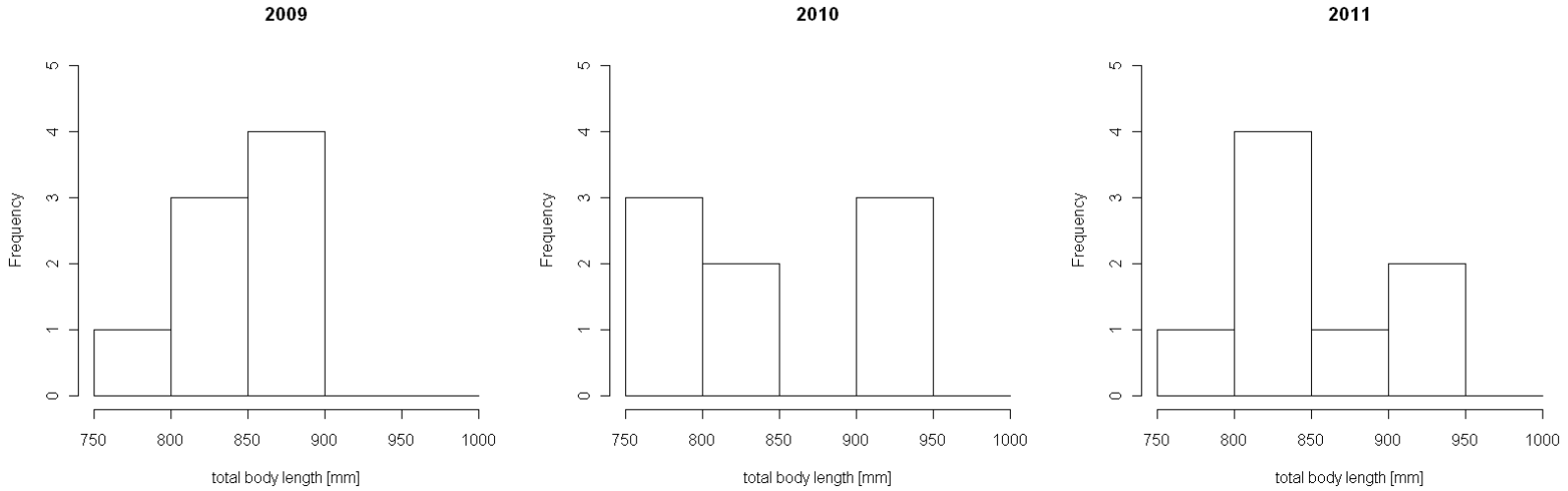


Fig. 4.2.2. Total body weight in g.

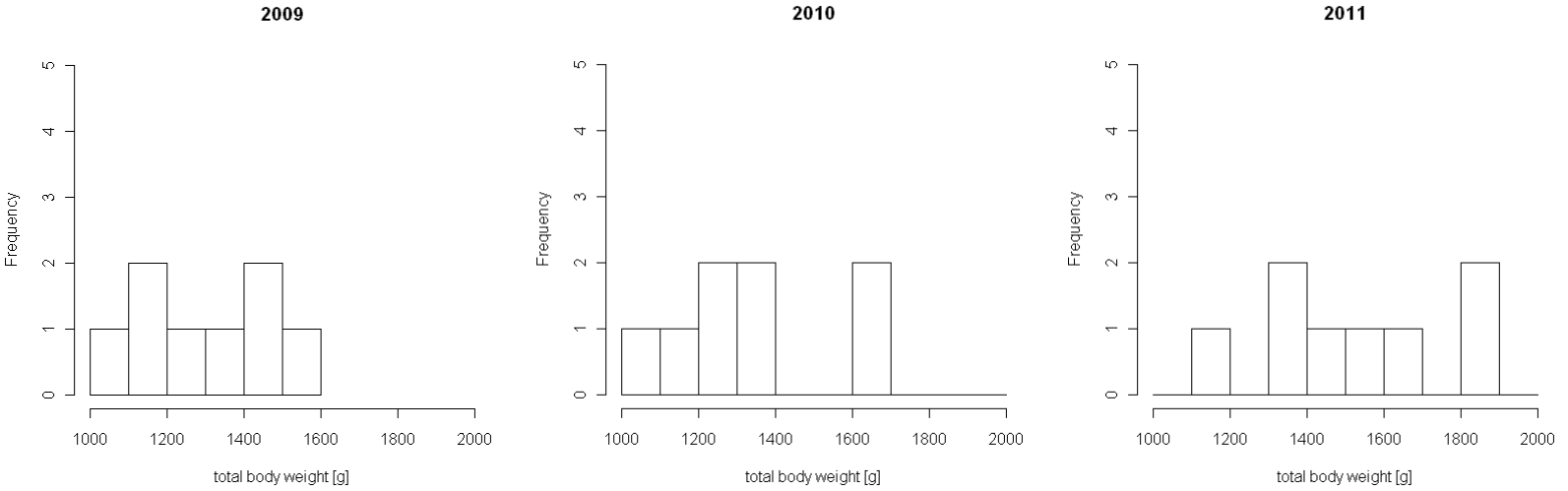


Fig. 4.2.3. Condition Factor

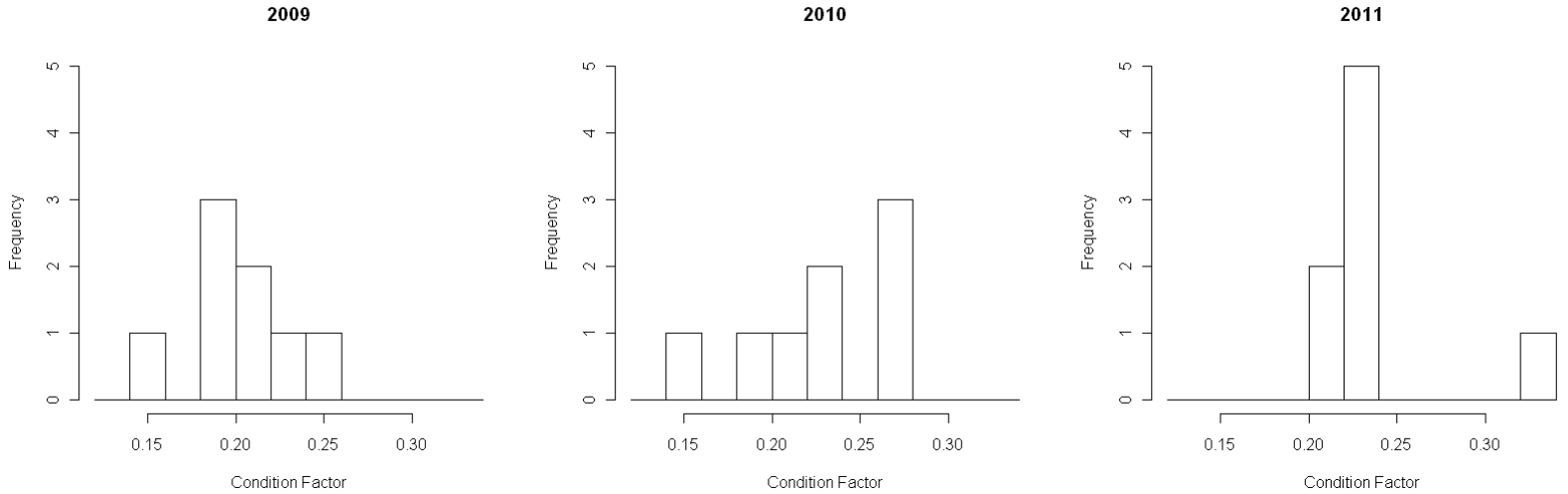


Fig. 4.2.4. Eye Index

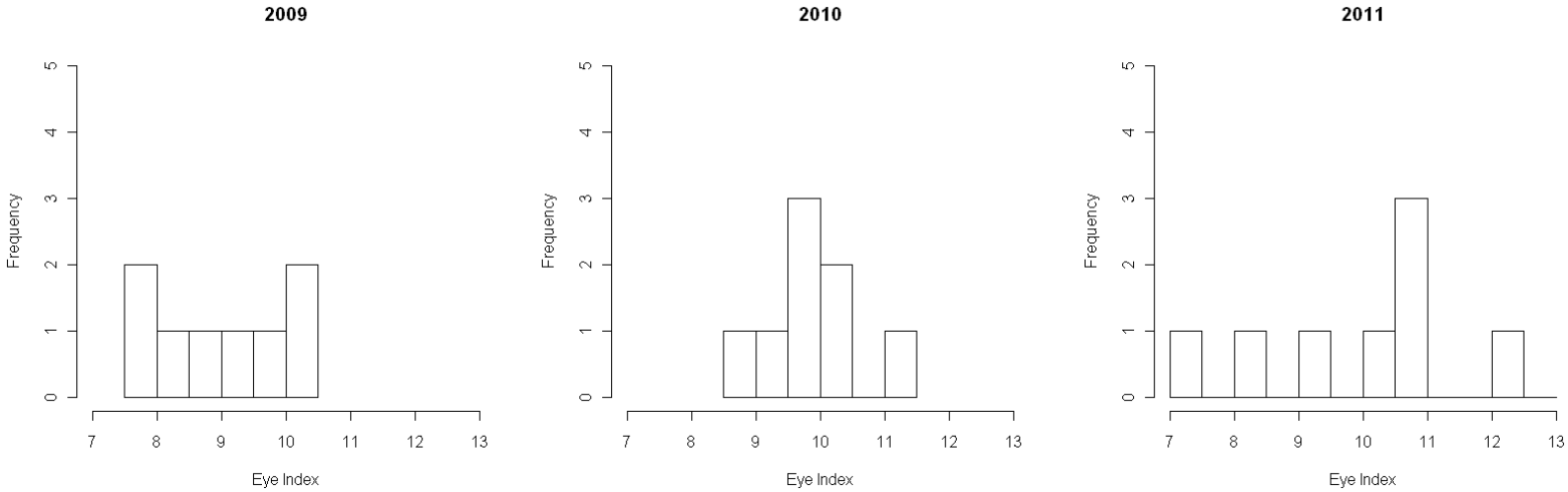


Fig. 4.2.5. Adequate Sampling Ratio

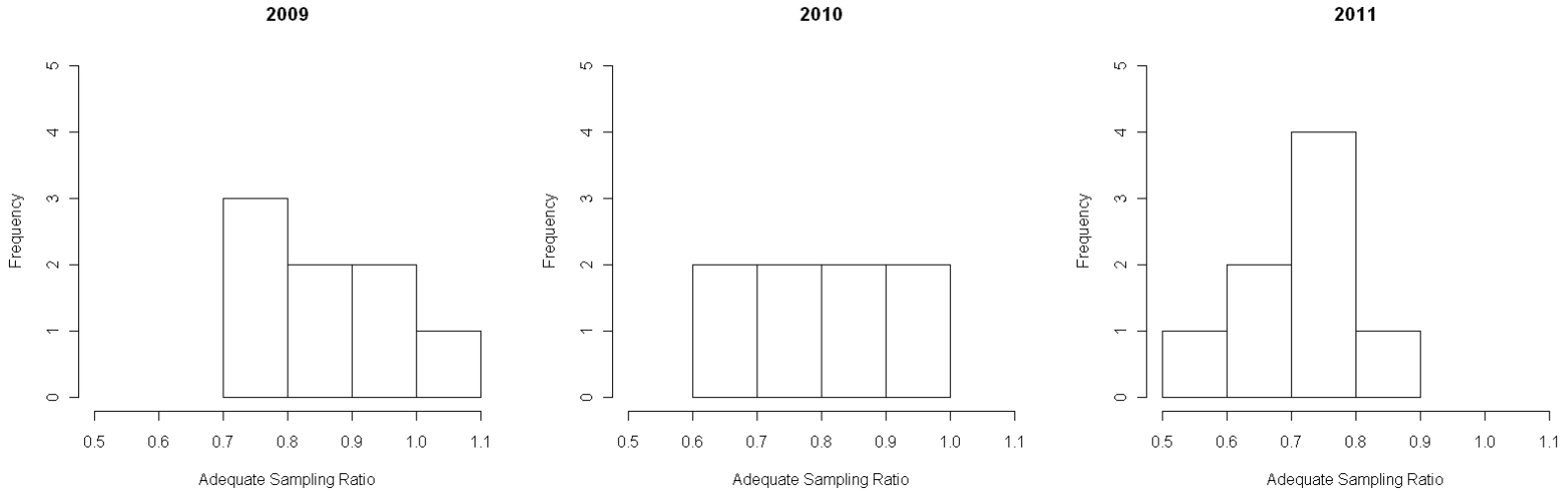
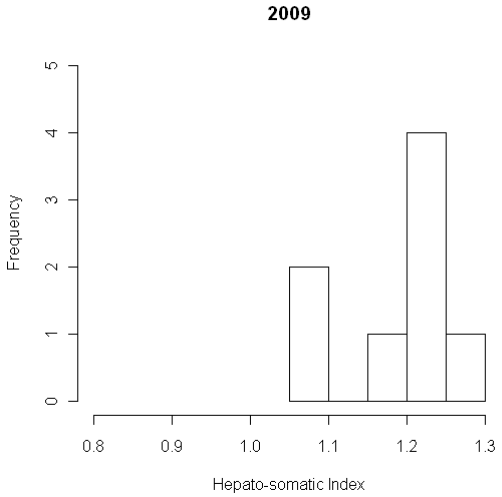
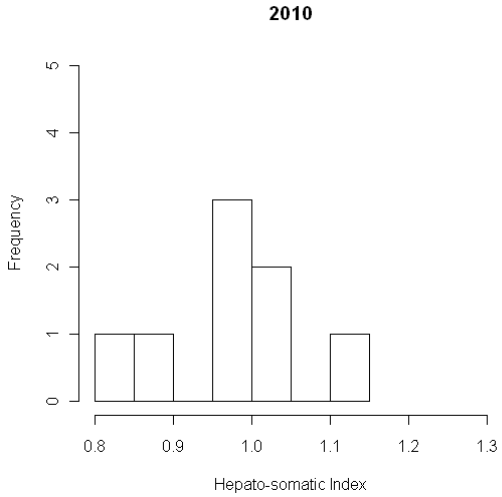


Fig. 4.2.6. Hepato-somatic Index

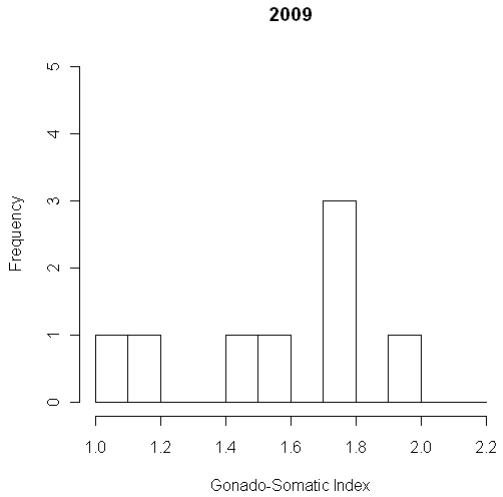


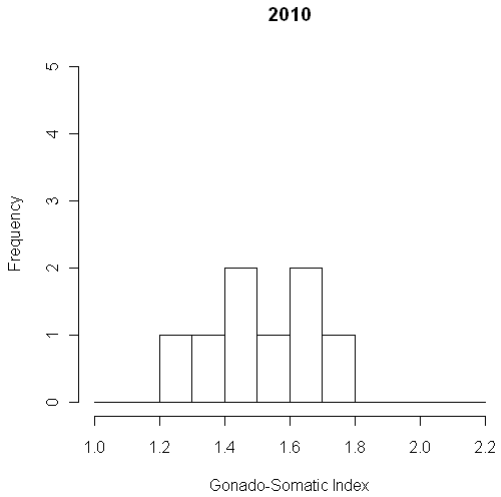


2011

DATA NOT AVAILABLE

Fig. 4.2.7. Gonado-somatic Index





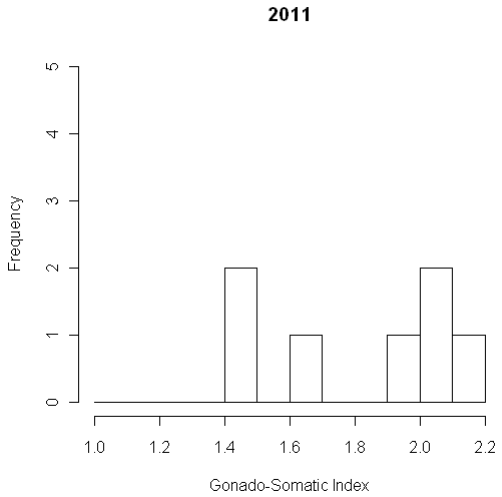


Fig. 4.2.8. Red Blood Cell count [erythrocytes $\cdot 10^6$ /mm^3]

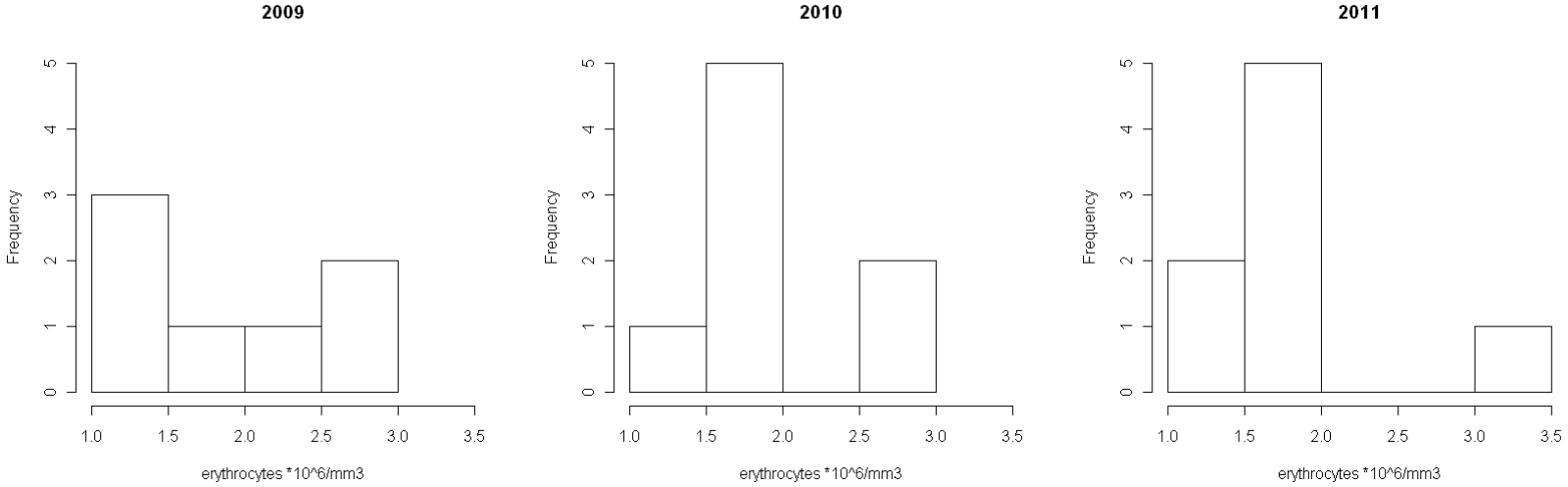


Fig. 4.2.9. White Blood Cell count [leukocytes $\cdot 10^3$ /mm^3]

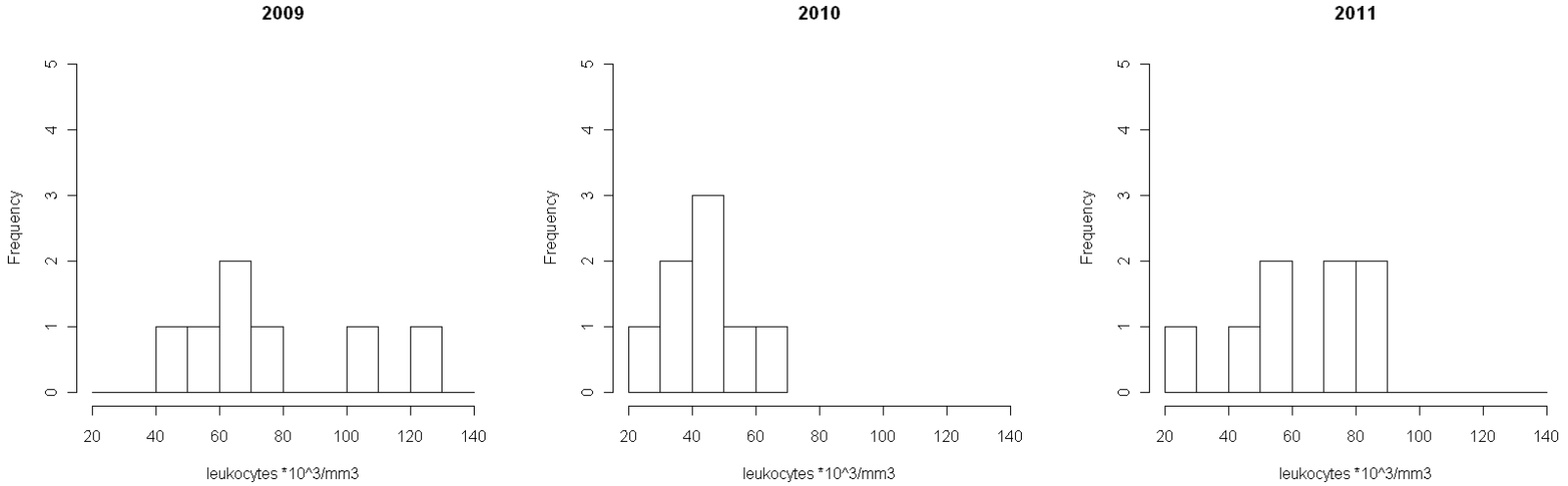


Fig. 4.2.10. Hematocrit [%]

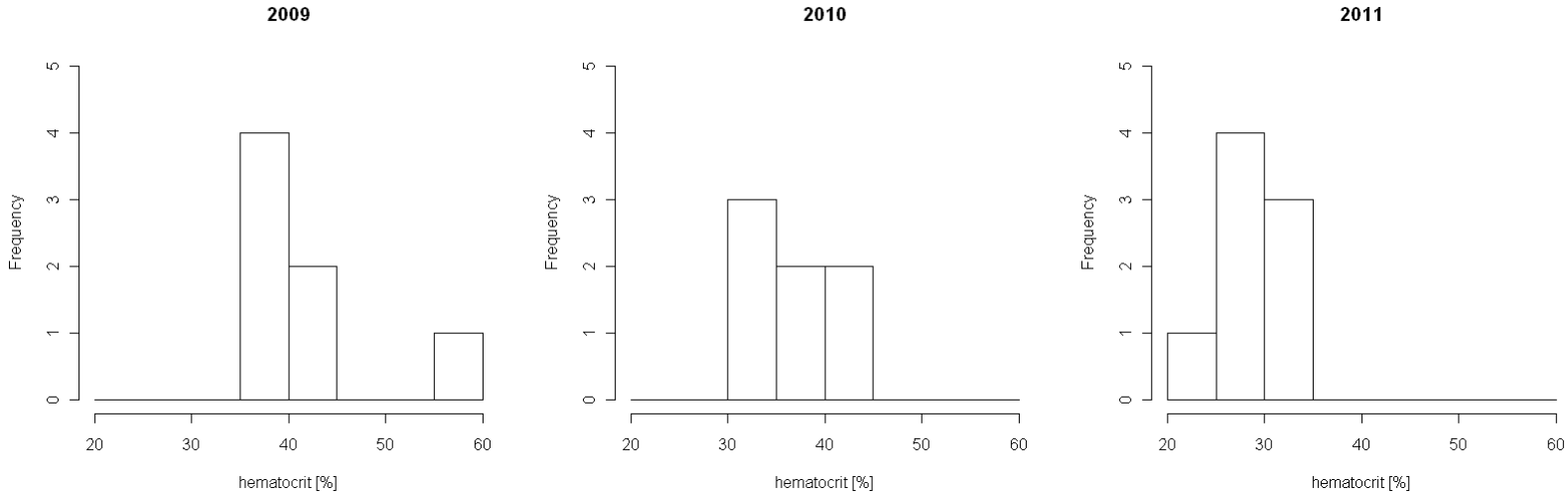


Fig. 4.2.11. Age determination – otoliths readings [years]

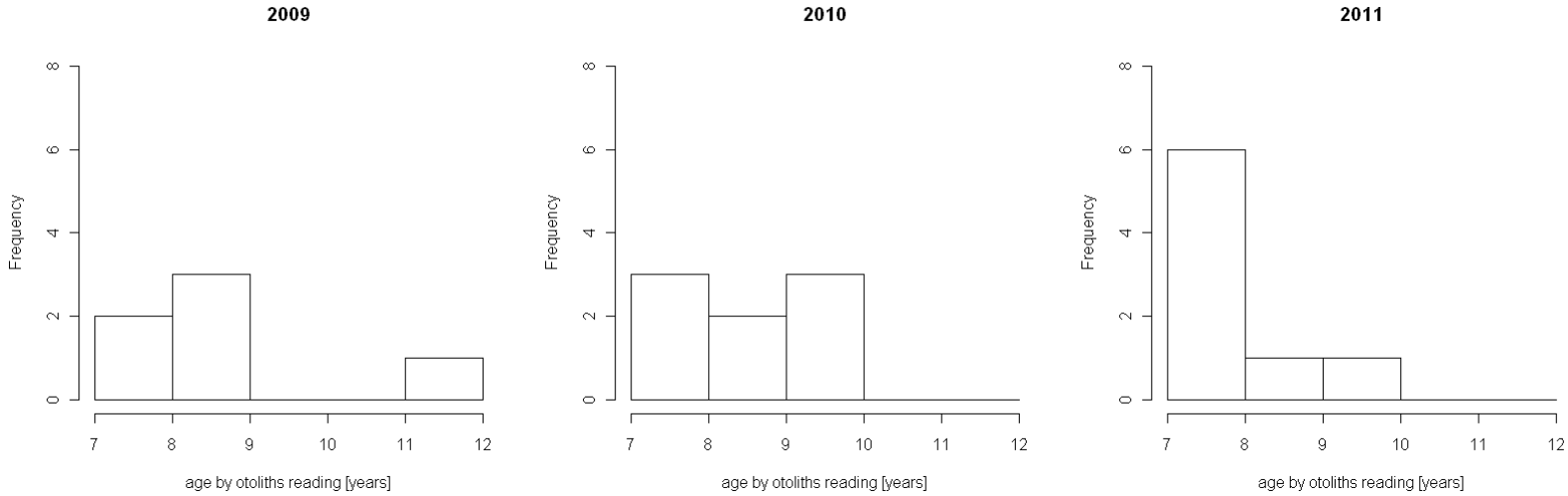
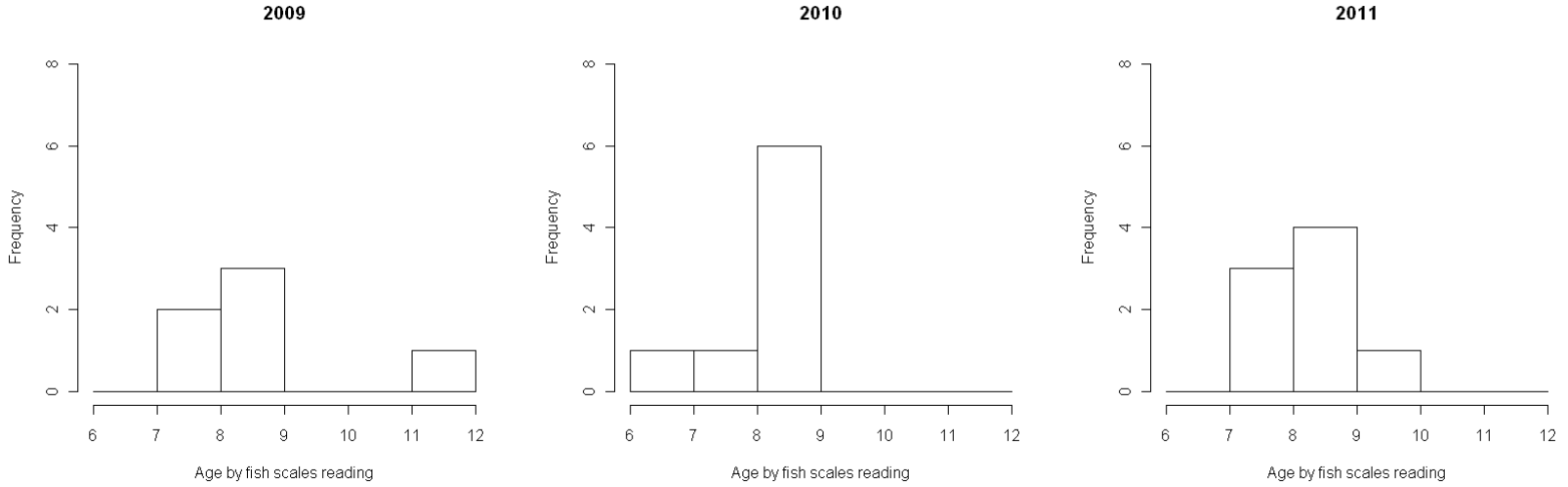


Fig. 4.2.12. Age determination – fish scales readings [years]



4.3 SEELF

4.3.1 SEELF calculation of Group A

On Group A was calculated only the SEELF A, using the following parameters:

	Range		*Threshold*	*Pi=1 if the value*
Parameter	*Minimum*	*Maximum*		*measured is*
Condition Factor			0.20	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<

Table 4.3.1. Values of ranges and thresholds assigned to SEELF A parameters. Units are omitted.

with the following evidences:

Year	N	SEELF A
2009	96	7.9±0.13
2010	100	9.6±0.05
2011	100	7.6±0.10

Table 4.3.2. SEELF A calculation on Group A. Data reported as mean±SE.

Sampling on population show moderate levels in 2009 and 2011, while in 2010 the best score was estimated, near to the top (define as 10.0). In 2010 the standard error (SE) is the lowest SE. The 2010 score is relevant if considered the high value of N (N=100).
For a comparison, historical data from other coastal lagoons are reported:

Lagoon, Year	N	SEELF A
P1, 2006	14	2.9±0.69
P2, 2006	15	3.3±0.58
VB, 2008	42	4.1±0.22
P3, 2008	43	4.1±0.35
P3, 2009	92	2.0±0.26
FB, 2009	27	0.5±0.19

Lagoon, Year	N	SEELF A
Comacchio, 2006	70	9.2±0.19
Comacchio, 2007	99	9.1±0.17
Comacchio, 2008	100	7.6±0.25
Comacchio, 2009	96	7.9±0.13
Comacchio, 2010	100	9.6±0.05
Comacchio, 2011	100	7.6±0.10

Tab. 4.3.3. SEELF A calculation on European eels caught in Comacchio and other coastal lagoons between 2006 and 201. Labels are used for identify private (P1, P2, P3) and public (VB, FB) lagoons. Data are reported as mean±SE.

Data elaborated with R Development Core Team (2011).

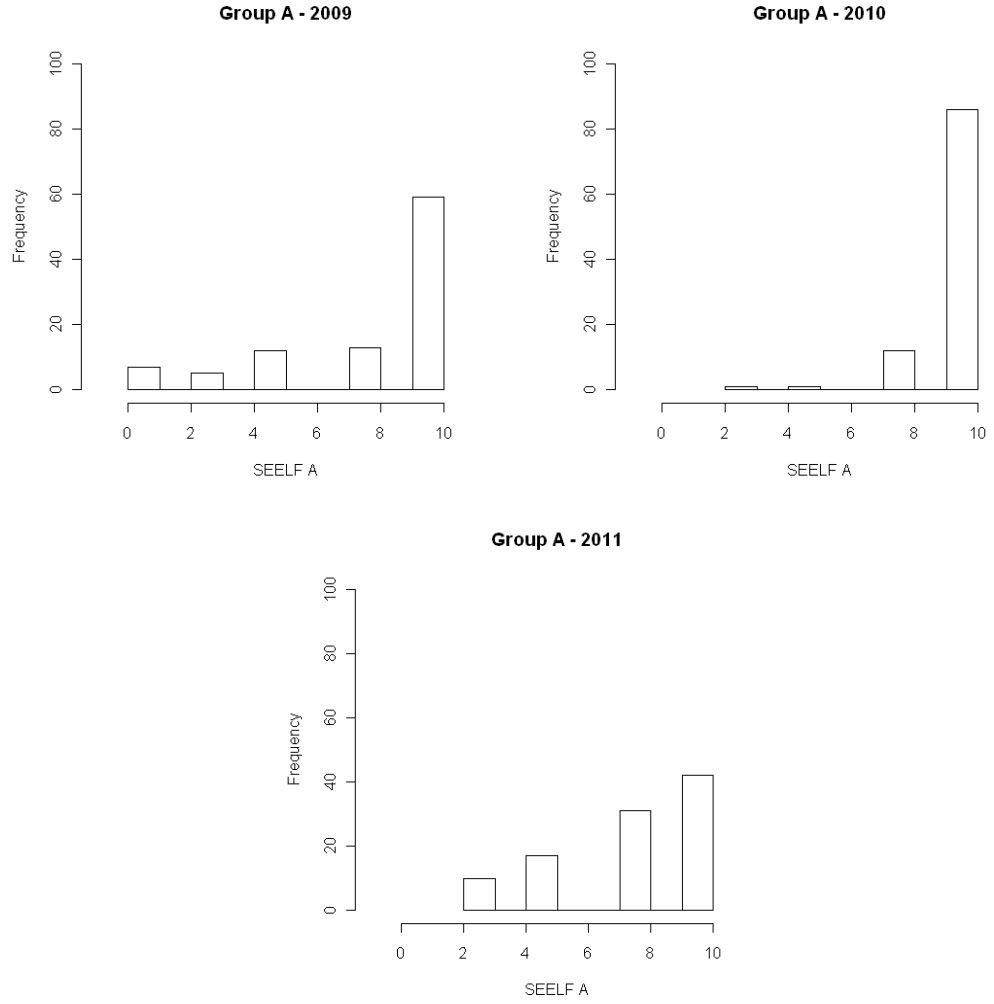


Figure 4.3.1. Graphs of SEELF A calculated on Group A.

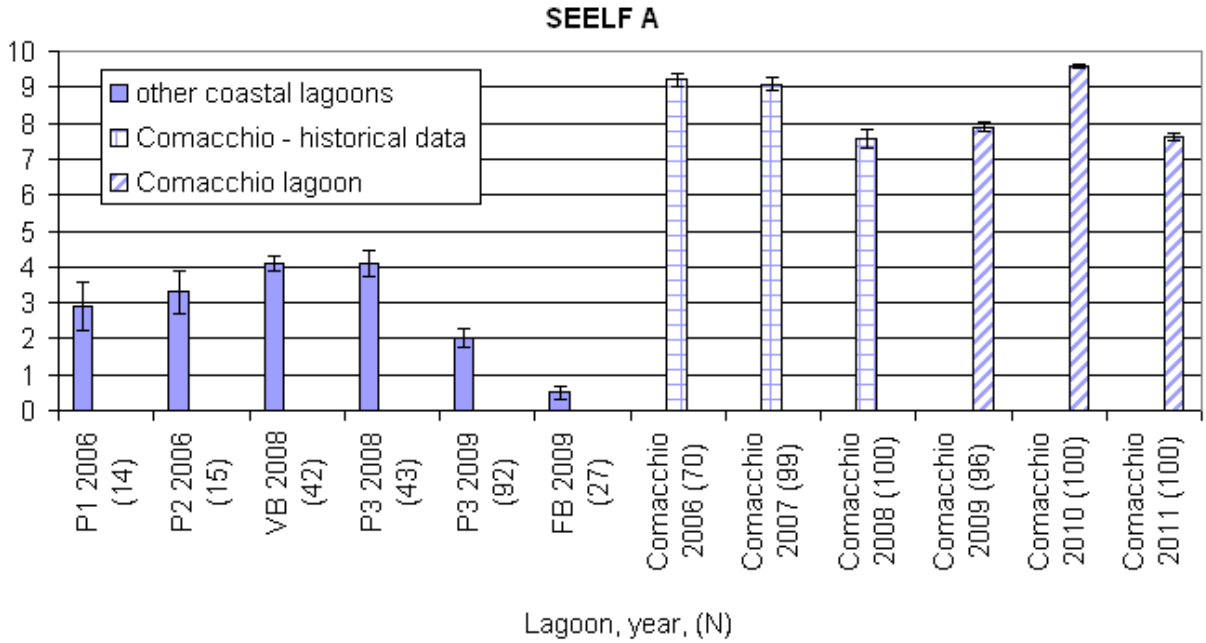


Figure 4.3.2. Graphs of SEELF A calculated on Group A, Comacchio lagoon from 2006 to 2008 and other coastal lagoons (from 2006 to 2009). Data are reported as mean±SE.

From 2006 the SEELF A index in Comacchio lagoon was >7.6, and in 3 years (2006, 2007, 2010) was >9. The smaller SEELF value is provided by FB, a lagoon open to coastal water. This area is more important as “nursery” than “breeding”. P1, P2, VB and P3 are closed lagoon, and the importance of capture-tool is shown by P3, than is >4 in 2008 and 2 in 2009. The SEELF A is calculated with general setting for healthy migrating eels, and the good score of the Comacchio lagoon is objective. A reference value of SEELF A for no-well managed coastal lagoons seems to be in a range from 3 to 4.

4.3.2 SEELF calculation of Group B

On Group B both SEELF A and SEELF B were calculated, using the following parameters:

Parameter	*Range*		*Threshold*	*Pi=1 if the value is*
	Minimum	*Maximum*		
Condition Factor			0.20	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<
Skin Evaluation			0.50	*
Hepato-somatic Index	1.00	1.50		Included
HT	35.0	45.0		Included
RBC	1,500,000	2,200,000		Included

Table 4.3.4. Values of ranges and thresholds assigned to SEELF's parameters. Units are omitted.

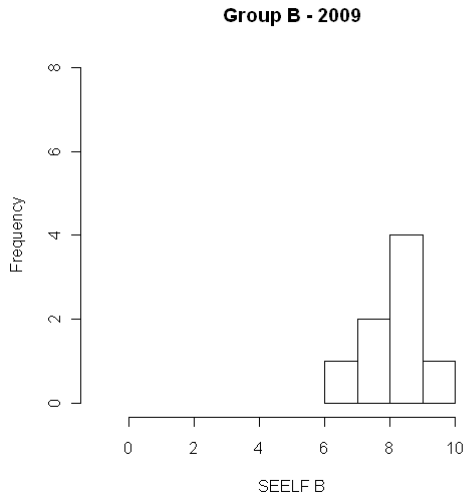
with the following evidences:

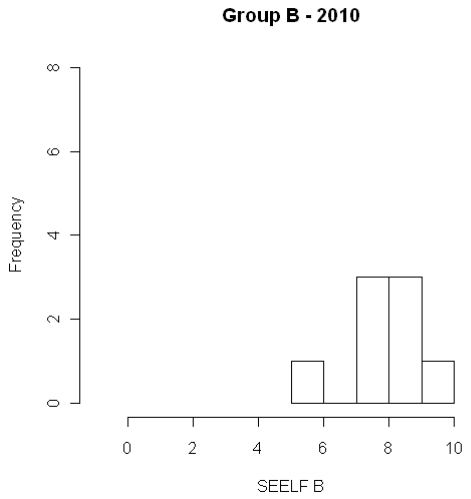
Year	N	SEELF A	SEELF B
2009	8	9.4±0.16	8.3±0.32
2010	8	9.4±0.16	8.0±0.42
2011	8	10.0±0.00	8.6±0.23

Table 4.3.5. SEELF A and SEELF B calculation on Group B. Data reported as mean±SE.

The SEELF A in 2009 and 2010 is exactly the same, while the value in 2011 is the highest value. All SEELF B values are lower that the respective SEELF A, because of the higher number of parameters used.

Data elaborated with R Development Core Team (2011).





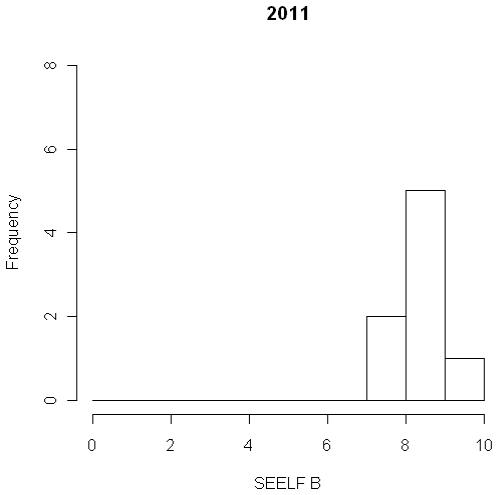


Figure 4.3.3. Graphs of SEELF B calculated on Group B.

The above calculations were performed using the references in table 4.3.4, defined as *a priori* setting. In order to evaluate the performance of the SEELF approach, different scenarios were evaluated, also comparing results with information produces by most important body indices:

	N	L	W	EI	CF	GSI
		[mm]	[g]			
2009	8	855±11.2	1310±55.5	9.02±0.37	0.210±0.008	1.56±0.11
2010	8	843±22.1	1363±78.9	9.9±0.27	0.23±0.02	1.51±0.06
2011	8	857±17.5	1535±84.9	10.0±0.53	0.24±0.01	1.82±0.10

Table 4.3.6. Main body indices of Group B. Data expressed as mean±SE.

Parameter: CF

For Condition Factor the variation should span to lower values, in order to define lowest value of CF threshold.

	SEELF B 2009	**SEELF B 2010**	**SEELF B 2011**
0.20	**8.3**	**8.0**	**8.6**
0.19	8.4	8.0	8.6
0.18	8.4	8.1	8.6
0.17	8.6	8.1	8.6
0.16	8.6	8.1	8.6
0.15	8.6	8.3	8.6

Table 4.3.7. Calculation of SEELF B, varying the Condition Factor.

Parameter: HSI

For Hepato-somatic Index the normal condition is a range. Livers of 2011 samples were used for other experiment and HIS was imposed as 1. The following tables summarize the most variations:

	SEELF B 2009	SEELF B 2010	SEELF B 2011
1.0÷1.5	**8.3**	**8.0**	NA
1.1÷1.5	8.0	7.7	
1.2÷1.5	7.8	7.5	
1.3÷1.5	7.0	7.5	

Table 4.3.8. Calculation of SEELF B, varying the hepato-somatic index.

No difference are reported for an increment of right value of range.

	SEELF B 2009	SEELF B 2010	SEELF B 2011
1.0÷1.5	**8.3**	**8.0**	NA
0.9÷1.4	8.3	8.4	
0.8÷1.3	8.3	8.8	
0.7÷1.2	7.5	8.8	

Table 4.3.9. Calculation of SEELF B, varying the hepato-somatic index.

Parameter: HT

For Hematocrit the normal condition is a range. The following tables summarize the most variations:

	SEELF B 2009	SEELF B 2010	SEELF B 2011
35÷45	**8.3**	**8.0**	**8.6**
36÷45	8.3	7.8	8.4
37÷45	8.0	7.8	8.4
38÷45	7.8	7.7	8.4
39÷45	7.8	7.5	8.4
40÷45	7.7	7.5	8.4

	SEELF B 2009	SEELF B 2010	SEELF B 2011
35÷45	**8.3**	**8.0**	**8.6**
34÷45	8.3	8.1	8.6
33÷45	8.3	8.1	8.9
32÷45	8.3	8.1	8.9

Table 4.3.10. Calculation of SEELF B, varying the hematocrit.

Increments of highest value of range does not produce changes in SEELF B.

	SEELF B 2009	SEELF B 2010	SEELF B 2011
35÷45	**8.3**	**8.0**	**8.6**
35÷45	8.3	8.0	8.6
35÷43	8.1	8.0	8.6
35÷42	8.1	8.0	8.6
35÷41	8.0	8.0	8.6
35÷40	8.0	7.7	8.6

	SEELF B 2009	SEELF B 2010	SEELF B 2011
35÷45	**8.3**	**8.0**	**8.6**
34÷44	8.3	8.1	8.6
33÷43	8.1	8.1	8.9
32÷42	8.1	8.1	8.9
31÷41	8.0	8.3	8.9
30÷40	8.0	8.0	8.9

Table 4.3.11. Calculation of SEELF B, varying the hematocrit

Parameter: RBC

For Red Blood Cell the normal condition is a range. The following tables summarize the most variations:

10^6	SEELF B 2009	SEELF B 2010	SEELF B 2011
1.4÷2.2	**8.3**	**8.0**	**8.6**
1.5÷2.2	8.3	8.0	8.6
1.6÷2.2	8.3	7.7	8.3
1,7÷2.2	8.1	7.5	8.1
1,8÷2.2	8.1	7.3	8.0

10^6	SEELF B 2009	SEELF B 2010	SEELF B 2011
1.4÷2.2	**8.3**	**8.0**	**8.6**
1.3÷2.2	8.4	8.1	8.6
1.2÷2.2	8.4	8.1	8.8
1.1÷2.2	8.8	8.1	8.8
1.0÷2.2	8.8	8.1	8.8

10^6	SEELF B 2009	SEELF B 2010	SEELF B 2011
1.4÷2.2	**8.3**	**8.0**	**8.6**
1.4÷2.3	8.4	8.0	8.6
1.4÷2.4	8.4	8.0	8.6
1.4÷2.5	8.4	8.0	8.6
1.4÷2.6	8.6	8.0	8.6
1.4÷2.7	8.6	8.3	8.6

10^6	SEELF B 2009	SEELF B 2010	SEELF B 2011
1.4÷2.2	**8.3**	**8.0**	**8.6**
1.3÷2.1	8.4	8.1	8.6
1.2÷2.0	8.4	8.1	8.8
1.1÷1.9	8.8	8.0	8.6
1.0÷1.8	8.8	8.0	8.4
0.9÷1.7	8.8	7.8	8.3

Table 4.3.12. Calculation of SEELF B, varying the red blood cell count.

Scenario 1 (modified values highlighted in bold):

	Range		*Threshold*	*Pi=1 if the value*
Parameter	*Minimum*	*Maximum*		*is*
Condition Factor			**0.18**	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<
Skin Evaluation			0.50	*
Hepato-somatic Index	**.90**	1.50		Included
HT	35.0	45.0		Included
RBC	1,500,000	2,200,000		Included

Table 4.3.13. Values of ranges and thresholds assigned to SEELF's parameters. Units are omitted.

And new scores for SEELF:

Year	N	SEELF A	SEELF B
2009	8	9.7±0.13	8.4±0.25
2010	8	9.7±0.13	8.6±0.23
2011	8	10.0±0.00	8.6±0.23

Table 4.3.14. Scenario 1 - SEELF A and SEELF B calculation on Group B.

Scenario 2 (modified values highlighted in bold):

Parameter	*Range*		*Threshold*	*Pi=1 if the value is*
	Minimum	*Maximum*		
Condition Factor			**0.18**	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<
Skin Evaluation			0.50	*
Hepato-somatic Index	**.90**	1.50		Included
HT	35.0	45.0		Included
RBC	**1,000,000**	**1,800,000**		Included

Table 4.3.15. Values of ranges and thresholds assigned to SEELF's parameters. Units are omitted.

And new scores for SEELF:

Year	N	SEELF A	SEELF B
2009	8	9.7±0.13	8.9±0.35
2010	8	9.7±0.13	8.6±0.23
2011	8	10.0±0.00	8.4±0.25

Table 4.3.16. Scenario 2 - SEELF A and SEELF B calculation on Group B.

Scenario 3 (modified values highlighted in bold):

Parameter	*Range*		*Threshold*	*Pi=1 if the value is*
	Minimum	*Maximum*		
Condition Factor			**0.18**	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<
Skin Evaluation			0.50	*
Hepato-somatic Index	1.00	1.50		Included
HT	**25.0**	**40.0**		Included
RBC	1,500,000	2,200,000		Included

Table 4.3.17. Values of ranges and thresholds assigned to SEELF's parameters. Units are omitted.

And new scores for SEELF:

Year	N	SEELF A	SEELF B
2009	8	9.7±0.13	8.1±0.19
2010	8	9.7±0.13	8.3±0.32
2011	8	10.0±0.00	9.5±0.18

Table 4.3.18. Scenario 3 - SEELF A and SEELF B calculation on Group B.

Scenario 4 (modified values highlighted in bold):

Parameter	*Range*		*Threshold*	*Pi=1 if the value is*
	Minimum	*Maximum*		
Condition Factor			0.20	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<
Skin Evaluation			0.50	*
Hepato-somatic Index	1.00	1.50		Included
HT	**25.0**	**40.0**		Included
RBC	**1,000,000**	**1,800,000**		Included

Table 4.3.19. Values of ranges and thresholds assigned to SEELF's parameters. Units are omitted.

And new scores for SEELF:

Year	N	SEELF A	SEELF B
2009	8	9.4±0.16	8.4±0.37
2010	8	9.4±0.16	8.1±0.33
2011	8	10.0±0.00	9.4±0.19

Table 4.3.20. Scenario 4 - SEELF A and SEELF B calculation on Group B.

Scenario 5 (modified values highlighted in bold):

	Range		*Threshold*	*Pi=1 if the value*
Parameter	*Minimum*	*Maximum*		*is*
Condition Factor			**0.18**	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<
Skin Evaluation			0.50	*
Hepato-somatic Index	**.90**	1.50		Included
HT	**25.0**	**40.0**		Included
RBC	**1,000,000**	2,200,000		Included

Table 4.3.21. Values of ranges and thresholds assigned to SEELF's parameters. Units are omitted.

And new scores for SEELF:

Year	N	SEELF A	SEELF B
2009	8	9.7±0.13	8.6±0.30
2010	8	9.7±0.13	8.9±0.35
2011	8	10.0±0.00	9.7±0.16

Table 4.3.22. Scenario 5 - SEELF A and SEELF B calculation on Group B.

Scenario 6 (modified values highlighted in bold):

	Range		*Threshold*	*Pi=1 if the value*
Parameter	*Minimum*	*Maximum*		*is*
Condition Factor			**0.18**	≥
Eye Index			6.50	≥
Silver Index			4	≥
Adequate Sampling Ratio			1.00	<
Skin Evaluation			0.50	*
Hepato-somatic Index	1.00	1.50		Included
HT	**25.0**	**40.0**		Included
RBC	**1,000,000**	**1,800,000**		Included

Table 4.3.23. Values of ranges and thresholds assigned to SEELF's parameters. Units are omitted.

And new scores for SEELF:

Year	N	SEELF A	SEELF B
2009	8	9.4±0.16	8.6±0.30
2010	8	9.4±0.16	8.3±0.32
2011	8	10.0±0.00	9.4±0.19

Table 4.3.24. Scenario 6 - SEELF A and SEELF B calculation on Group B.

Year	*N*	*SEELF B*							*GSI*
		0	1	2	3	4	5	6	
2009	8	103.9	98.2	103.6	98.1	103.8	96.5	**103.8**	**103.3**
2010	8	100.0							100.0
2011	8	107.8	100.0	98.2	115.1	115.4	108.8	**113.2**	**111.9**

Table 4.3.25. Comparison between SEELFB scores and GSI values, expressed as percentage of the lowest (SEELF B 2010=100%).

If compared with GSI, a setting *a posteriori* could be choose as Scenario no.6, where condition factor is the minimum report in literature for a good status, hematocrit a wider range and RBC is a range centred on $1.4 \cdot 10^6$ erythrocytes/mm^3.as cited by Ghittino (1984) for farmed eels.

	Initial hypothesis – Scenario 0			*Scenario 6*			
Parameter	*Range*		*Threshold*	*Range*		*Threshold*	*Pi=1 if the value is*
	Min	*Max*		*Min*	*Max*		
CF			0.20			**0.18**	≥
EI			6.50			6.50	≥
SI			4			4	≥
ASR			1.00			1.00	<
SE			0.50			0.50	*
HIS	1.00	1.50		1.00	1.50		Included
HT	35.0	45.0		**25.0**	**40.0**		Included
RBC	1,500,000	2,200,000		**1,000,000**	**1,800,000**		Included

Table 4.3.26. Comparison between the setting of SEELF B (modified values highlighted in bold).

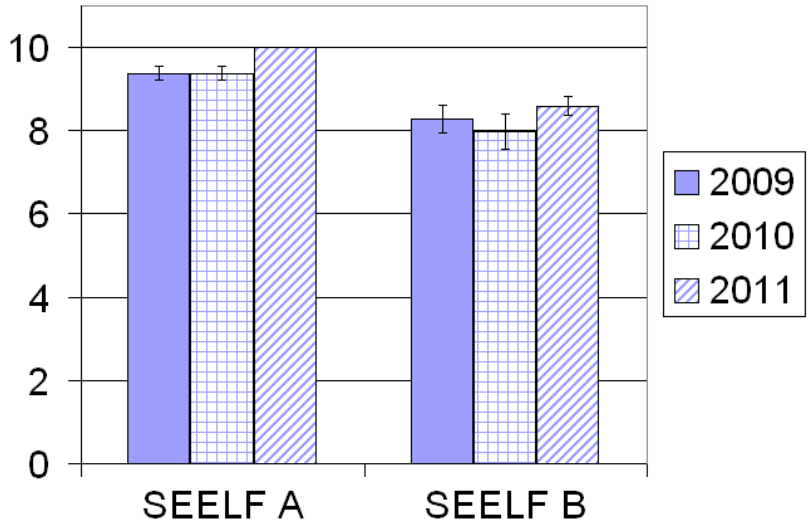


Figure 4.3.4. Graphs of both SEELF A and SEELF B calculated on Group B.

4.4 Calibration between otoliths and fish scales readings

In 4.2 the age determinations are reported as biological ages. Biological age is the estimated age for a fish, calculated as:

Biological age of eel = otolith reading + marine nucleus value (MNV)

So MNV = Biological age of eel - otolith reading

And Biological age of eel = fish scale reading + age factor (AF)

So AF = Biological age of eel - fish scale reading

In fact, both marine nucleus value (MNV) and age of development of fish scale (AF), cannot be estimated in details. Tesch (2003) report high variability of MNV and AF, but during this work, a comparison between the two readings was possible.

Lecture of otoliths is the most precise method for obtaining information on fish age, while use of scales is less precise but has the advantage of not being destructive. In otolith reading, the environmental conditions influences the recognizing of the zero band and the stock management (i.e. marking, stocking) can affect precision of age determination (ICES, 2011). Fish scales in *A. anguilla* is not proved to be an age-dependent process (Tesch, 2003), and the difference between the number of otolith and scale annuli becomes greater as age increases Rasmussen (1952). The variability was reduced using an homogeneous (silver eels similar in size) sample, as shown by the table below.

Year	N	L	W	EI	CF	Age otolith	Age fish scale	Otolith Length	Otolith Growth*
		[mm]	[g]			[year]	[year]	[mm]	[mm/y]
2009	6	847±13.4	1287±53.1	8.70±0.45	0.22±0.01	7.2±0.6	5.2±0.5	3.92±0.15	0.43±0.01
2010	8	843±22.1	1363±78.9	9.87±0.27	0.23±0.02	7.0±0.4	4.6±0.3	3.99±0.08	0.43±0.01
2011	8	857±17.5	1535±84.9	9.97±0.53	0.24±0.01	6.4±0.3	4.8±0.3	4.07±0.07	0.49±0.02
Mean	*22*	849±13.1	1405±60.3	9.75±0.31	0.23±0.01	6.8±0.2	4.8±0.2	3.96±0.06	0.45±0.01

Tab. 4.4.1. Morphometrical estimates, age determination, otoliths length and otolith growth rate in silver eels. Data are reported as mean±SE. *Calculated with age determination by otoliths readings.

The following values were assumed:

MNV = 2

AF = 4

The following table summarize the experimental results:

Year	N	Age otolith	Biological Age otolith	Age fish scale	Biological Age fish scale	Δ age $_{otolith}$ – age $_{fish\ scale}$
		[year]	[year]	[year]	[year]	[year]
2009	6	7.2±0.6	9.2±0.6	5.2±0.5	9.2±0.5	2.0±0.00
2010	8	7.0±0.4	9.0±0.4	4.6±0.3	8.6±0.3	2.4±0.26
2011	8	6.4±0.3	8.4±0.3	4.8±0.3	8.8±0.3	1.6±0.26
Mean	*22*	*6.8±0.2*	*8.8±0.2*	*4.8±0.2*	*8.8±0.2*	*2.0±0.15*

Tab. 4.4.2. Age readings and biological ages estimated by otoliths and fish scales in *A. anguilla*. Data are reported as mean±SE.

This makes possible to calculate a Δ factor, defined as the difference between the values of age by otolith reading and fish scale reading.

The age by otolith reading was used for comparison to several parameters, such as otolith growth (defined as otolith length/age by otolith, fig.4.4.1a), age by fish scale reading (fig.4.4.1b), otolith length (fig.4.4.1c) and total body length (fig.4.4.1d). The relationships, calculated on N=22 (including samples 2009, 2010, 2011) show a moderate linearity (R^2=0.57) for *a* and *b*, but not for *c* and *d*.

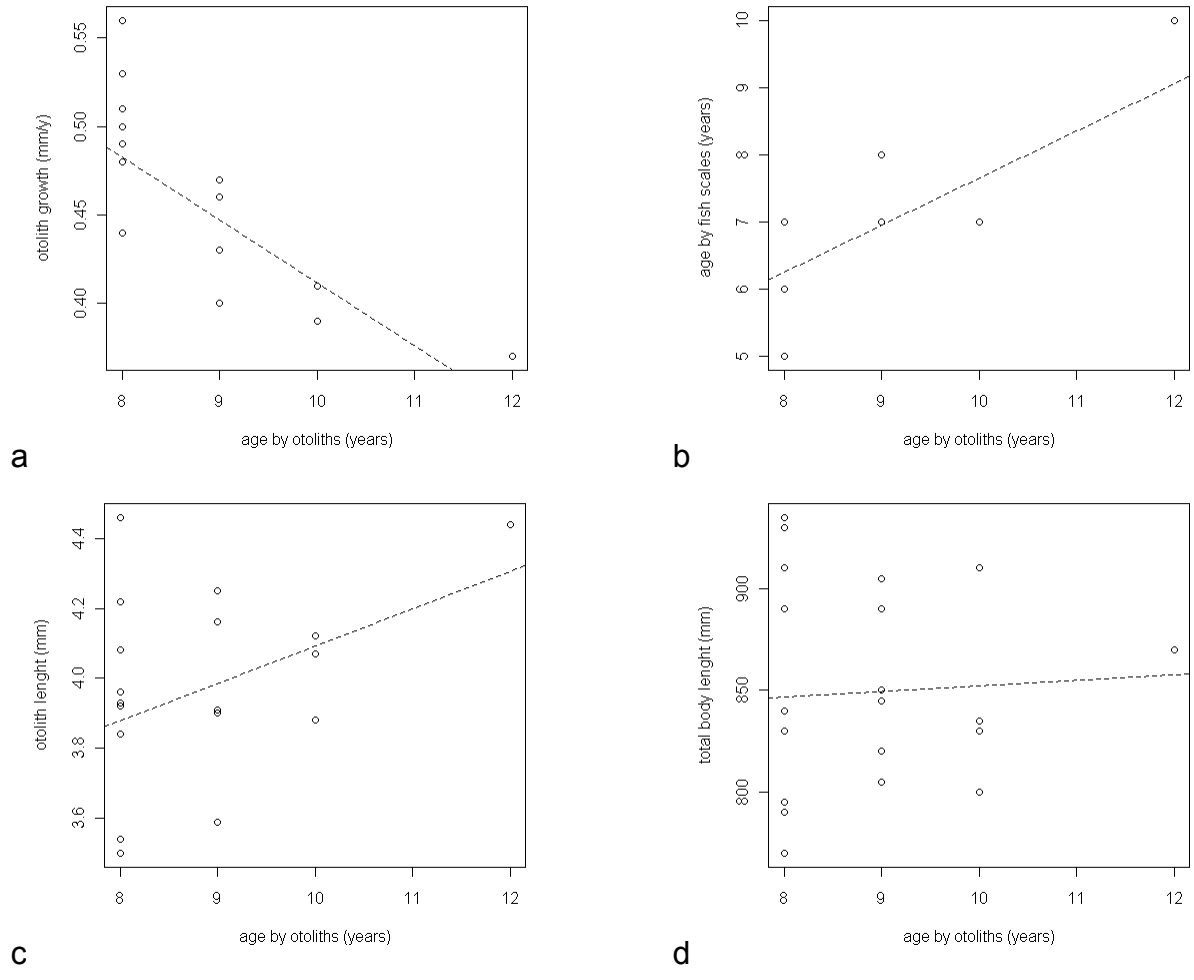


Figure 4.4.1. Relationship between morphometric parameters and age by otolith reading (otolith growth-a, age by fish scale reading-b, otolith length-c and total body length-d)

The Δ factor span from 1 to 3, as reported in figure 4.4.2:

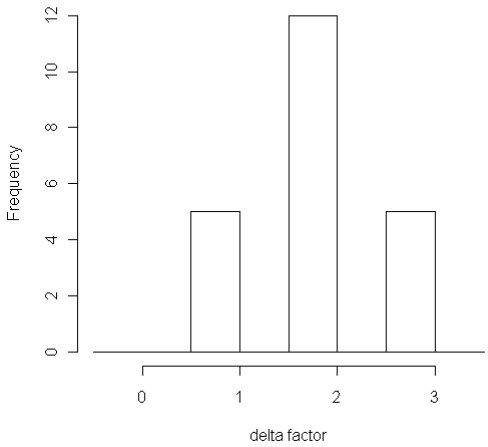


Figure 4.4.2. The Δ factor (2009, 2010, 2011; N=22)

Data elaborated with R Development Core Team (2011).

4.5 ChE assays

4.5.1 ChE forms characterization

4.5.1.1 ChE activities, substrate preference and tissue distribution

Eel average (N=12) weight was 1311±47.5 g, total length was 84.3±1.0 cm and were all female at fourth stage of silvering. Eel ChE activities in skeletal muscle, brain, plasma and liver at three concentrations (0.05, 0.5 and 5 mM) of different substrates (ASCh, PSCh and BSCh) are shown in figure 4.5.1 and ChE forms ratio are summarized in table 4.5.1.

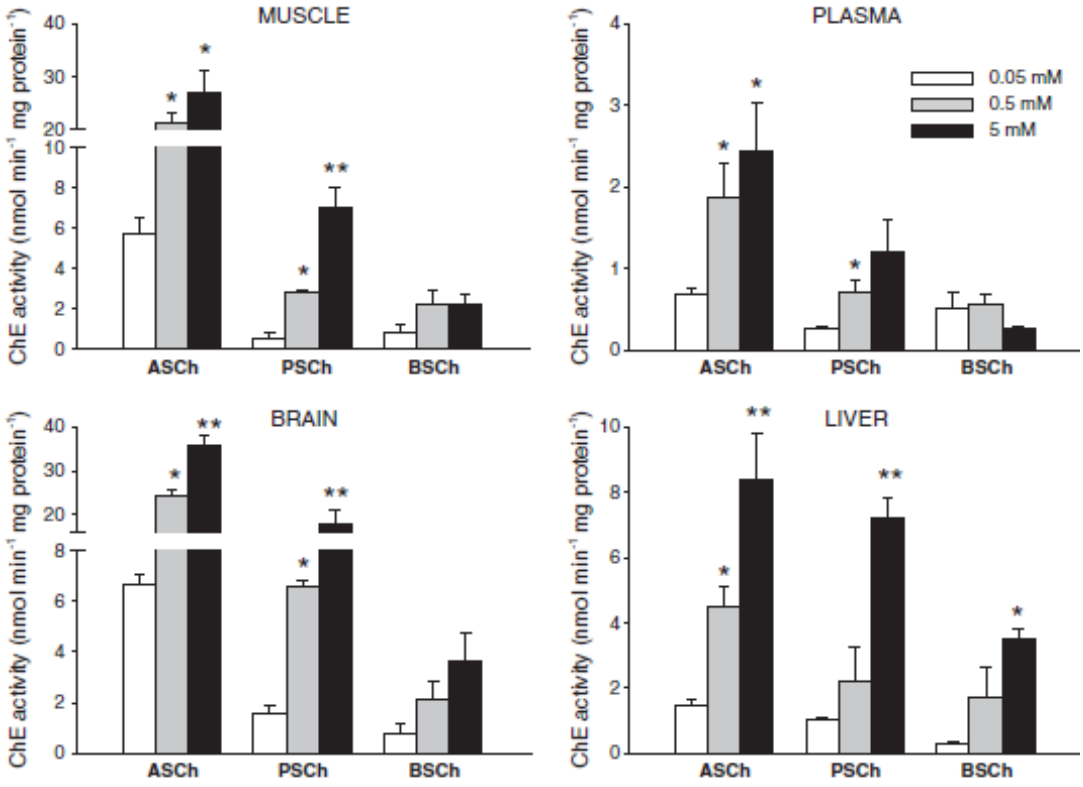


Figure 4.5.1. Eel ChE activities in skeletal muscle, brain, plasma and liver at three concentrations of different substrates: ASCh, PSCh, and BSCh. Different Y axis scaling is used to allow proper reading of data. Data are expressed as the mean±SE from 3 individuals (n=3), analysed independently and in triplicate. For each substrate *P<0.05 vs 0.05 mM substrate; **P<0.05 vs 0.05 and 0.5 mM substrate.

Tissue	AChE activity	BChE activity	PChE activity
Brain	73.8	19.8	6.39
Muscle	80.6	10.7	8.70
Liver	53.4	26.1	20.5
Plasma	60.0	22.5	17.6

Table 4.5.1. Eel AChE, BChE PChE activities detected in brain, skeletal muscle, liver and plasma of silver eels expressed as % of total ChE activity.

For all tissues and substrates, except for BSCh hydrolysis in plasma, enzyme activities increased with increasing substrate concentrations. At the different concentrations,

hydrolysis of ASCh was always higher than that of PSCh, which in turn was higher than that of BSCh. ASCh hydrolysis was then separately evaluated in tissues from a larger sample, i.e. 12 silver eels. ASCh hydrolysis in brain and muscle was about 5-fold higher than in liver and about 10-fold higher than in plasma. Results obtained at 0.5 mM substrate are summarized in table 4.5.2. Features of samples are compared with previous reports dealing with ChE activities in *A. anguilla* (Table 4.5.4).

4.5.1.2 Kinetic parameters

The effects of substrate concentration on brain and muscle enzyme activities were assayed independently in each of the 12 individuals using ten ASCh concentrations in the range of 0.001–10 mM (figure 4.5.2). ChE activity followed the Michaelis–Menten kinetic, and apparent Km and Vmax values for ASCh hydrolysis were as reported in figure 4.5.2 inset.

Tissue	ChE activity [nmol min^{-1} mg $protein^{-1}$]
Brain	23.2±1.03
Muscle	19.6±2.49
Liver	4.17±0.93
Plasma	2.16±0.32

Table 4.5.2. Enzyme activity was assessed in the presence of 0.5 mM ASCh. Data are expressed as the mean±SE from 12 individuals (n=12).

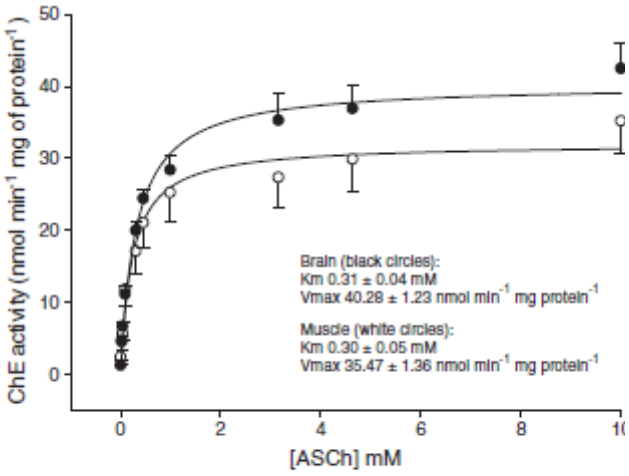


Figure 4.5.2. Michaelis–Menten plot describing ChE kinetic parameters. Data are expressed as mean±SE (N=12), analysed independently and in triplicate.

4.5.1.2 Use of specific inhibitors

Further experiments were carried out in the presence of selective inhibitors of AChE (BW284c51) and BChE (iso-OMPA); the inhibition by the non-selective ChE inhibitor eserine was also assessed (figure 4.5.3). IC50 values are as reported in figure 4.5.3 inset. Strong and dose-dependent inhibition of ASCh hydrolysis was obtained in the presence of

eserine and BW284c51, confirming the predominance of specific AChE activity in brain and muscle from silver eels. No inhibition was obtained in the presence of iso-OMPA.

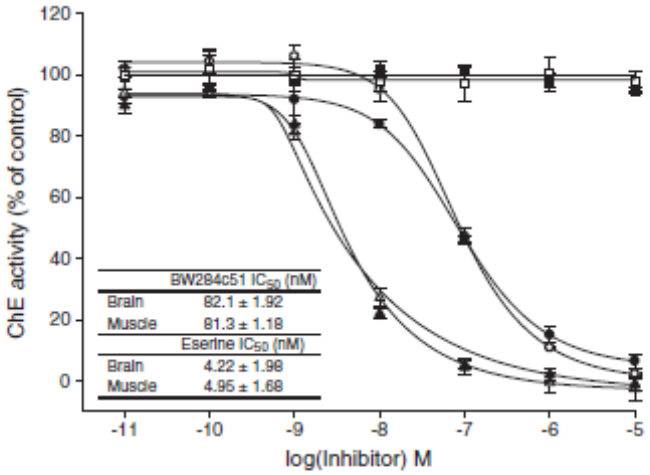


Figure 4.5.3. Percent inhibition of ChE activity by BW284c51 (circles) iso-OMPA (squares) and eserine (triangles) evaluated in eel brain (black) and skeletal muscle (white); Basal levels are 22.8±1.1 and 19.1±1.9 nmol·min^{-1}·mg of protein^{-1}, for brain and skeletal muscle, respectively.
Data are expressed as mean±SE (N=3), analyzed independently and in triplicate.

4.5.1.4 In vitro ChE inhibition by pesticides

In vitro effects of four pesticides, representative of the organophosphate (A) and the carbamate (B) classes selected among those widely used world wide, were assessed on ChE activities in eel brain and muscle (figure 4.5.4). Calculated IC50 values are shown in table 4.5.3, listed in comparison with other reports. ChE activities in brain and muscle were significantly affected by in vitro exposure to pesticides with the following order of potency: carbofuran>carbaryl>chlorpyrifos≥diazinon.

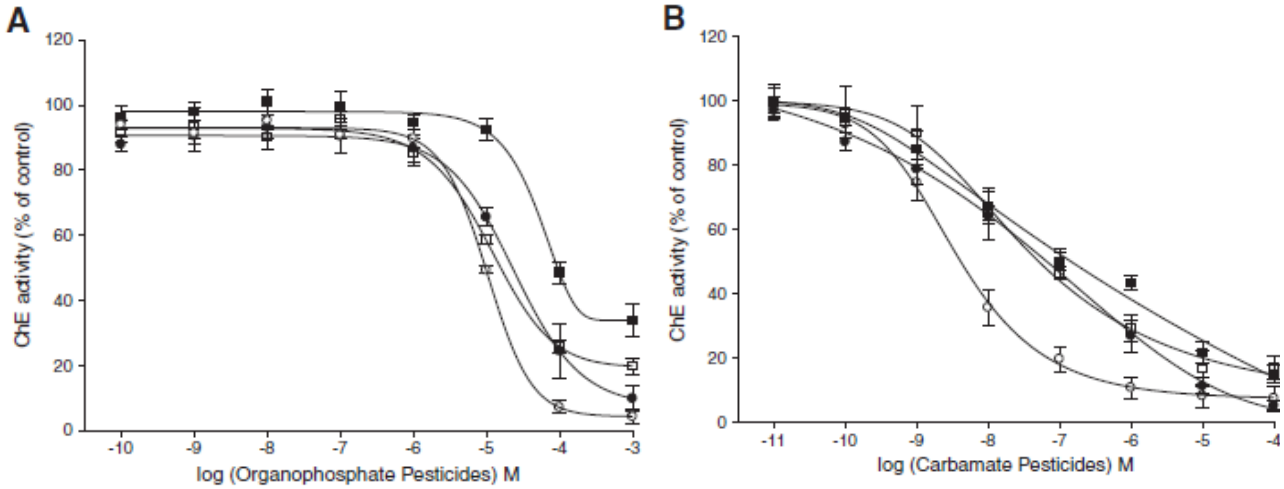


Figure 4.5.4. Percent inhibition of ChE activity by organophosphate (A) and carbamate (B) classes of pesticides. (A) Chlorpyrifos (white symbols) and diazinon (black symbols) analyzed in brain (squares) andmuscle (circles). (B) Carbofuran (white symbols) and carbaryl (black symbols) analysed in brain (squares) and muscle (circles). Basal levels are 23.3±0.9 and 20.1±1.4 nmol·min^{-1}·mg of protein^{-1}, for brain and skeletal muscle, respectively. Data are expressed as mean±SE (N=6), analyzed independently and in triplicate.

Pesticides	Fish	Reference	IC_{50} (M)
Carbaryl	*A. anguilla*	Valbonesi et al., 2011	4.1×10^{-7}
	*A. anguilla**	Valbonesi et al., 2011	1.2×10^{-7}
	C. macropomum	Assis et al., 2010	3.4×10^{-5}
	C. gariepinus	Mdegela et al., 2010	3.0×10^{-9}
Carbofuran	*A. anguilla*	Valbonesi et al., 2011	2.4×10^{-8}
	*A. anguilla**	Valbonesi et al., 2011	3.4×10^{-9}
	C. carpio	Dembélé et al., 2000	4.1×10^{-7}
	C. macropomum	Assis et al., 2010	9.2×10^{-7}
Chlorpyrifos	*A. anguilla*	Valbonesi et al., 2011	1.3×10^{-5}
	*A. anguilla**	Valbonesi et al., 2011	1.0×10^{-5}
	C. carpio	Dembélé et al., 2000	8.1×10^{-4}
	C. macropomum	Assis et al., 2010	7.6×10^{-6}
Diazinon	*A. anguilla*	Valbonesi et al., 2011	9.5×10^{-5}
	*A. anguilla**	Valbonesi et al., 2011	2.3×10^{-5}
	C. carpio	Dembélé et al., 2000	1.9×10^{-5}
	C. macropomum	Assis et al., 2010	No effect
	C. gariepinus	Mdegela et al., 2010	1.5×10^{-7}
	D. rerio	Keizer et al., 1995	2.0×10^{-5}
	O. mykiss	Keizer et al., 1995	2.5×10^{-6}
	P. reticulata	Keizer et al., 1995	7.5×10^{-6}

Table 4.5.3. ChE inhibition in fish tissues treated in vitro with organophosphate or carbamate pesticides. ChE activities were evaluated in fish brain. *ChE activities were evaluated in white skeletal muscle.

Reference	Length	Weight	Condition Factor	Stage	ChE activities* A – [µmol/min/g w w] B – [µmol/min/g prot]					Country	Water	Period of capture	Stunning or anesthesia
	[cm]	[g]			Brain	Muscle	Liver	Plasma					
Ceròn et al., 1996	16–20	20–30		Y	6.30±0.70			1.24±0.17	A	S	T		MS222
Sancho et al., 2000	16–20	20–30		Y	6.96±0.27			1.58±0.29	A	S	T		MS222
Fernández-Vega et al., 2002	16–20	20–30		Y	9.61±0.78	16.48±3.37			A	S	T		MS222
Fernández-Vega et al., 2002	16–20	20–30		Y	0.05±0.00	0.17±0.02			B	S	T		MS222
Gravato et al., 2010	31.8±1.1	59.86±6.60	0.16±0.00	Y	19.00±0.55				B	P	S	Winter	
Gravato et al., 2010	27.7±0.5	28.42±2.49	0.12±0.00	Y	25.54±0.80				B	P	S	Winter	
Gravato et al., 2010	28.4±0.7	45.69±4.27	0.17±0.01	Y	33.16±1.22				B	P	S	Winter	
Guimarães et al., 2009	35.1±1.13	83.9±11.69	0.17±0.003	Y	15				B	P	S	Autumn	
Guimarães et al., 2009	34.5±1.18	70.6±9.11	0.15±0.004	Y	17				B	P	S	Autumn	
Guimarães et al., 2009	32.3±1.01	62.3±7.04	0.17±0.005	Y	19				B	P	S	Autumn	
Ferenczy et al., 1997		300÷615			308.8±22.7	143.7±18.7	37.3±8.4		B	H	F		
This work, as reported by Valbonesi et al., 2011	**84.2±1.0**	**1311±47.5**	**0.22±0.00**	**S**	**23.2±1.03**	**19.6±2.49**	**4.17±0.93**	**2.16±0.32**	**B**	**I**	**B**	**Autumn**	**Ice**

Table 4.5.4. Comparison of ChE activities and main features in European eel from different reports. *In the different reports, enzyme activities are reported either as ChE or AChE activities. However they were evaluated through the same methodology (Ellman et al., 1961), using acetylthiocholine as the substrate. Stage:Y-yellow, S-silver. Country: S-Spain, P-Portugal, H=Hungary, I-Italy. Water: T-tap (fish farm), S-seawater (river), F-freshwater (lake), B-brackish (lagoon).

4.5.2 AChE assay

AChE activity was estimated in brain, skeletal muscle, liver and plasma of silver eels, in order to evaluate its magnitude and capability to use this parameter for future investigations. The sampling strategy aims to select the most appropriated technique to be used in food processing and environmental monitoring. First, this means that the target organ/tissue have to be easy to sampling. Because of this requirement and AChE activity, the brain and plasma should be discharged (brain is time-expensive and plasma is rarely sampled “in field” without corruption or sample) and liver sampling destroy a large part of fish carcass. The table 4.5.5 summarizes the results.

Tissue	AChE activity (nmol min^{-1} mg protein $^{-1}$)				
	2009	2010	2011	Mean	Valbonesi et.al. 2011
Brain	22.8±0.12	NA	NA*	---	23.2 ± 1.03
Muscle	20.5±0.97	23.4±3.55	22.7±3.30	22.7±1.99	19.6 ± 2.49
Liver	1.8±0.22	NA	NA**	---	4.17 ± 0.93
Plasma	1.8±0.22	NA	NA*	---	2.16 ± 0.32

Table 4.5.5. AChE activity detected in brain, skeletal muscle, liver and plasma of silver eels. Data are reported as mean±SE. (NA-not available, *sample used for genetic investigation, ** sample used for metabolism investigation)

During the 2009 sample the target tissue was defined and in 2010 and 2011 the assays were performed only using the skeletal muscle. The experiments were performed according to animal welfare, but without use of substance for anaesthesia that could change the expected result. The AChE activity in skeletal muscle is estimated very closed to that in brain. The average on N=22 is comparable to value reported by Valbonesi et al (2011) and by Fernàndez-Vega et al. (2002). Valbonesi et al studied silver eels from Comacchio lagoon, while Fernàndez-Vega et al. studied yellow farmed eels very different in body size. Fernàndez-Vega et al. declare the use of MS222 for anaesthesia and this could affect the result of assays, as discussed in 3.1. The AChE activity estimated in brain (only 2009) is comparable to results of Gravato et al. (2010), measured in yellow eels captured in winter in an estuary.

Table 4.5.6 summarizes the experimental data with previous reports.

Reference	Length	Weight	Condition Factor	Stage	ChE activities* A – [µmol/min/g w w] B – [µmol/min/g prot]					Country	Water	Period of capture	Stunning or anesthesia
	[cm]	[g]			Brain	Muscle	Liver	Plasma					
Ceròn et al., 1996	16–20	20–30		Y	6.30±0.70			1.24±0.17	A	S	T		MS222
Sancho et al., 2000	16–20	20–30		Y	6.96±0.27			1.58±0.29	A	S	T		MS222
Fernández-Vega et al., 2002	16–20	20–30		Y	9.61±0.78	16.48±3.37			A	S	T		MS222
Fernández-Vega et al., 2002	16–20	20–30		Y	0.05±0.00	0.17±0.02			B	S	T		MS222
Gravato et al., 2010	31.8±1.1	59.86±6.60	0.16±0.00	Y	19.00±0.55				B	P	S	Winter	
Gravato et al., 2010	27.7±0.5	28.42±2.49	0.12±0.00	Y	25.54±0.80				B	P	S	Winter	
Gravato et al., 2010	28.4±0.7	45.69±4.27	0.17±0.01	Y	33.16±1.22				B	P	S	Winter	
Guimarães et al., 2009	35.1±1.13	83.9±11.69	0.17±0.003	Y	15				B	P	S	Autumn	
Guimarães et al., 2009	34.5±1.18	70.6±9.11	0.15±0.004	Y	17				B	P	S	Autumn	
Guimarães et al., 2009	32.3±1.01	62.3±7.04	0.17±0.005	Y	19				B	P	S	Autumn	
Ferenczy et al., 1997		300÷615			308.8±22.7	143.7±18.7	37.3±8.4		B	H	F		
Valbonesi et al., 2011	84.2±1.0	1311±47.5	0.22±0.00	S	23.2±1.03	19.6±2.49	4.17±0.93	2.16±0.32	B	I	B	Autumn	Ice
This work, 2009	**85.9±0.8**	**1300±45.2**	**0.21±0.01**	**S**	**22.8±0.12**	**20.5±0.97**	**1.8±0.22**	**1.8±0.22**	**B**	**I**	**B**	**Autumn**	**Ice**
This work, 2009-2010-2011	**84.4±0.8**	**1358±34.7**	**0.23±0.01**	**S**		**22.7±1.99**			**B**	**I**	**B**	**Autumn**	**Ice**

Table 4.5.6. Comparison of ChE activities and main features in European eel from different reports. Data are reported as mean±SE. *In the different reports, enzyme activities are reported either as ChE or AChE activities. However they were evaluated through the same methodology (Ellman et al., 1961), using acetylthiocholine as the substrate. Stage:Y-yellow, S-silver. Country: S-Spain, P-Portugal, H=Hungary, I-Italy. Water: T-tap (fish farm), S-seawater (river), F-freshwater (lake), B-brackish (lagoon).

4.6 Blood biochemistry

The blood sampled by silver eels was evaluated in blood chemistry, in order to estimate a characteristic features to assign to migrant eels. Thus, these experiments are part of a wide evaluation of the health status of eels captured in the Comacchio lagoon.

Because of references are not available for silver eels, these experimental results are milestones for the characterization of the eel stock in the Comacchio lagoon. Although this is a sampling of N=24, an evaluation on mean levels in different annual sample, as well as confidential bars, can provide an useful information for further analyses.

		Units	2009	2010	2011
GLI	Glycaemia	(mg/dL)	321±30.2	132±8.0	114±27.6
UREA		(mg/dL)	8.21±0.73	5.73±0.62	7.07±0.75
LDH		(U/L)	84726±41170	4733±1263	1503±310
AST		(U/L)	1453±392	287±46.7	207±50.7
ALT		(U/L)	18.86±5.38	1.63±0.33	1.75±0,37
FA		(U/L)	26.14±4.17	22.13±1.32	25.13±3.40
CK-NAK	creatine kinase	(U/L)	299848±113297	16843±4201	17291±9823
CA		(mg/dL)	10.74±0.41	9.99±0.09	9.97±0.06
P		(mg/dL)	10.57±2.55	6.29±0.31	5.26±0.22
Mg		(mg/dL)	5.19±0.88	4.13±0.36	3.06±0.19
COL	Cholesterol	(mg/dL)	594±37.2	579±14.4	589±32.0
TRI	Triglycerides	(mg/dL)	507±62.8	607±46.9	729±77.3
PT		(g/dL)	4.65±0.12	4.47±0.08	4.46±0.13
ALB	Albumin	(g/dL)	1.12±0.042	1.00±0.020	0.97±0.022
Na		(mmol/L)	158±1.3	155±0.7	158±0.9
K		(mmol/L)	4.59±0.28	4.05±0.31	4.13±0.15
Cl		(mmol/L)	126±3.9	130±0.83	134±0.8
R. A/G			0.32±0.01	0.29±0.00	0.28±0.01

Table 4.6.1. Blood chemistry in silver eels. Data are reported as mean±SE.

Values of glycaemia (GLI), LDH, CK, ALT and AST show significant differences between 2009 and 2010-2011 samples, probably due to stress condition during transport. Other parameters don’t show significant differences.

Metabolites

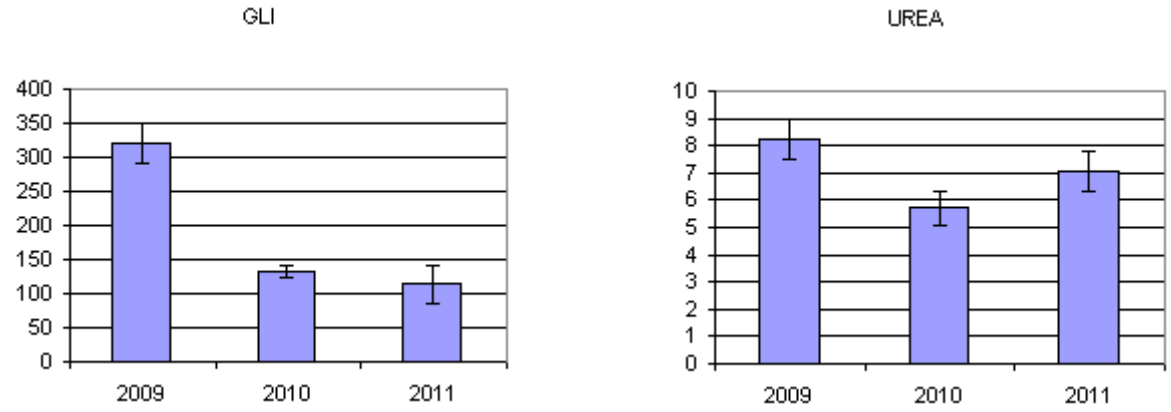


Figure 4.6.1. Metabolites on silver eels [mg/dL].

Enzymes

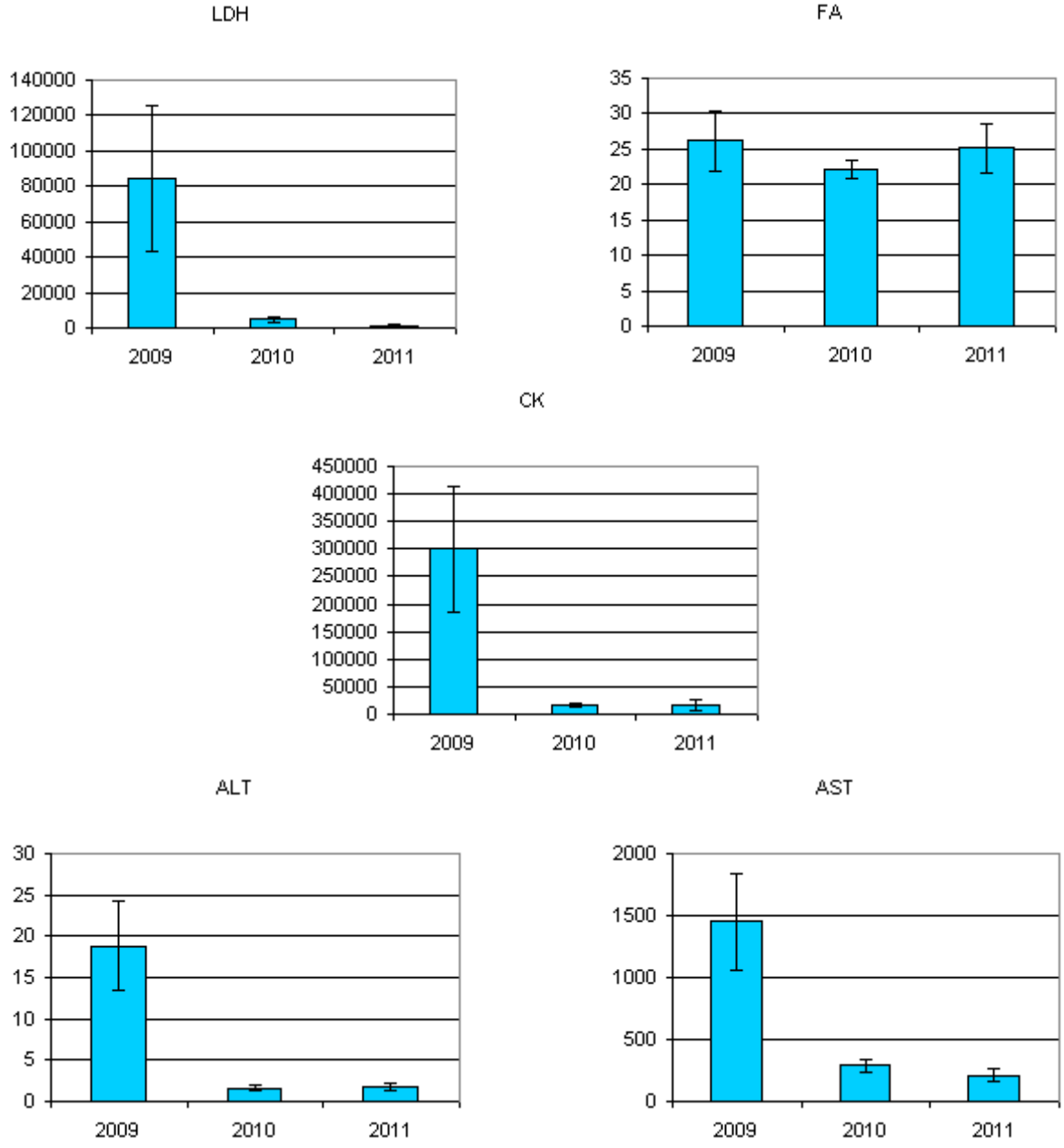


Figure 4.6.2. Enzymes on silver eels [U/L].

Electrolytes

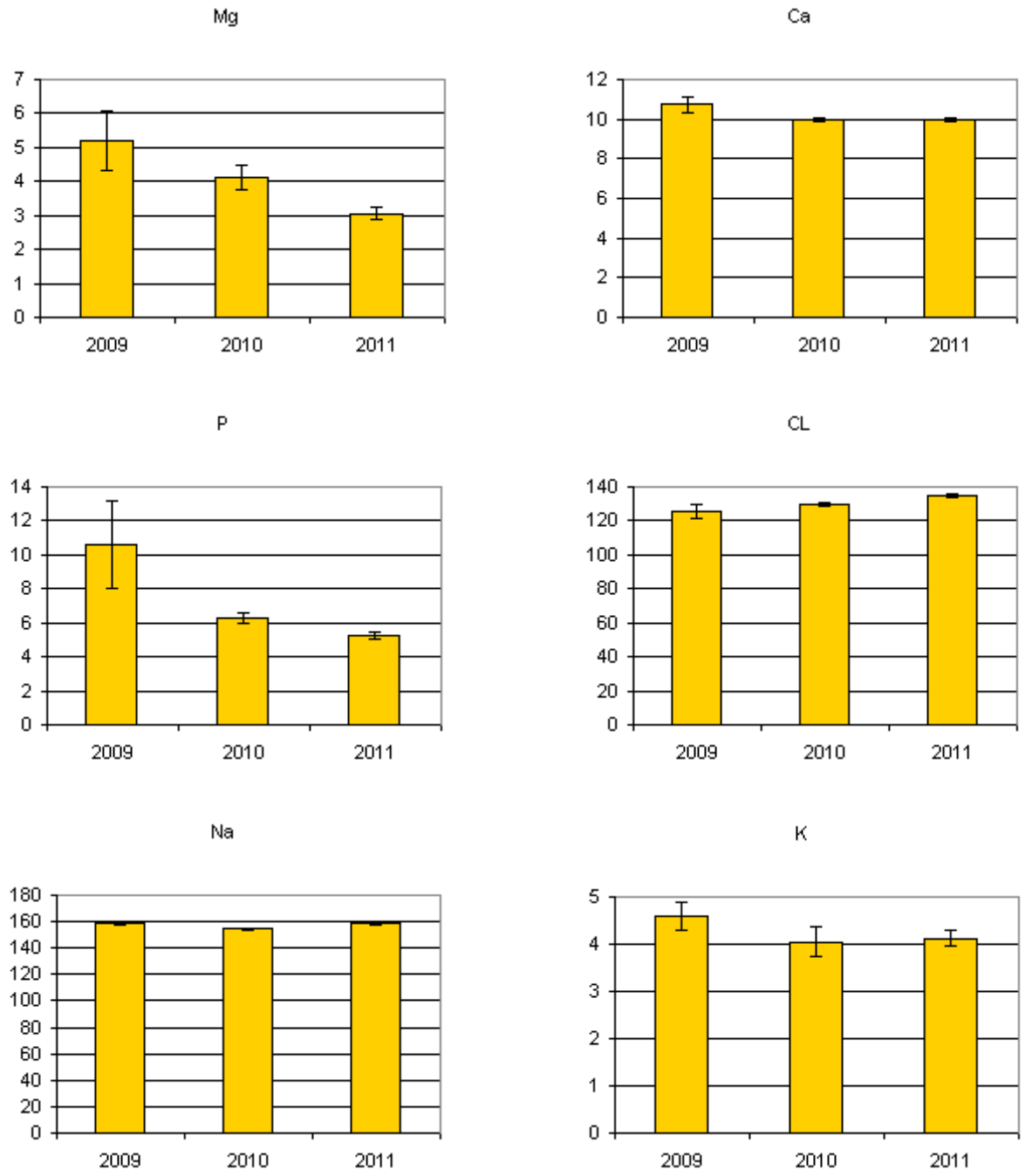


Figure 4.6.3. Electrolytes on silver eels [units: Mg, Ca, P in mg/dL; Cl, Na, K in mmol/L].

Proteins

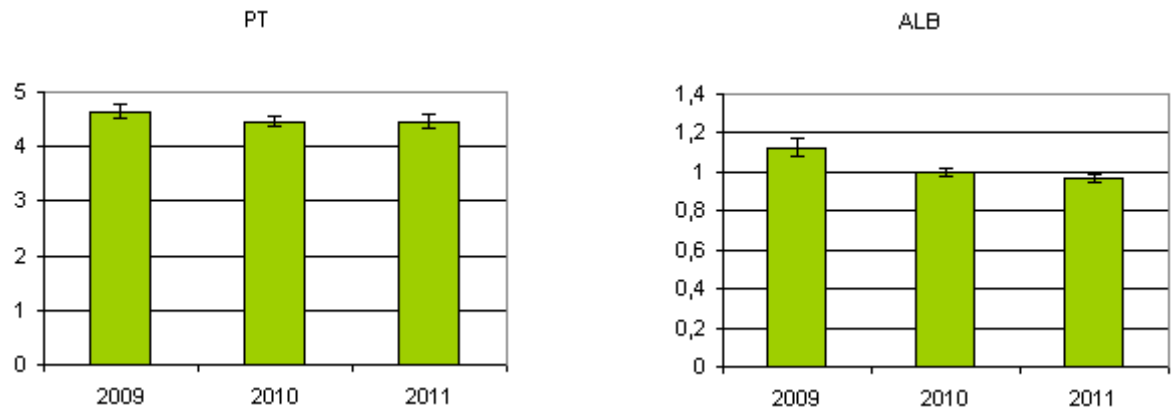


Figure 4.6.4. Protein on silver eels [g/dL].

Lipids

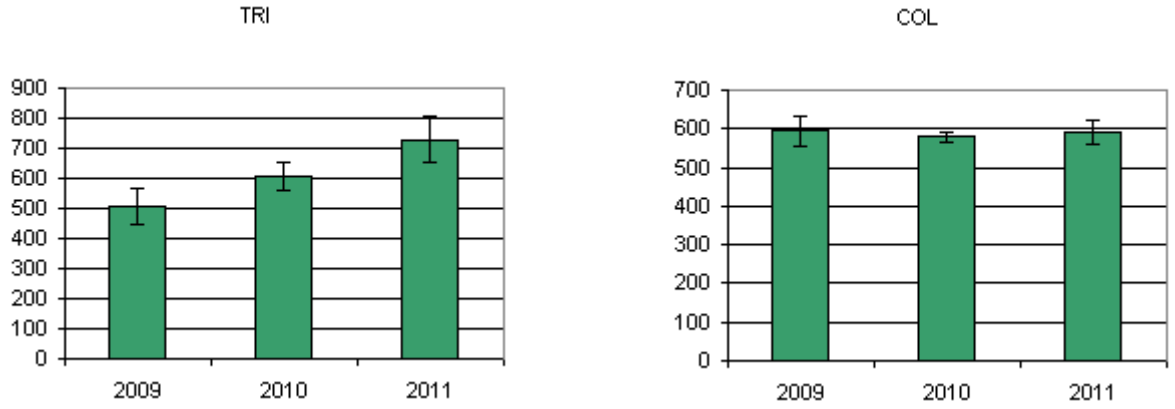


Figure 4.6.5. Lipids on silver eels [mg/dL].

4.7 Genetic investigation

The samples are located in different points of the phylogenetic tree (figure 4.7.1), closed to other specimen captured in Denmark, France, Greece, Ireland, Lithuania, Sweden and Italy (figure 4.7.2).

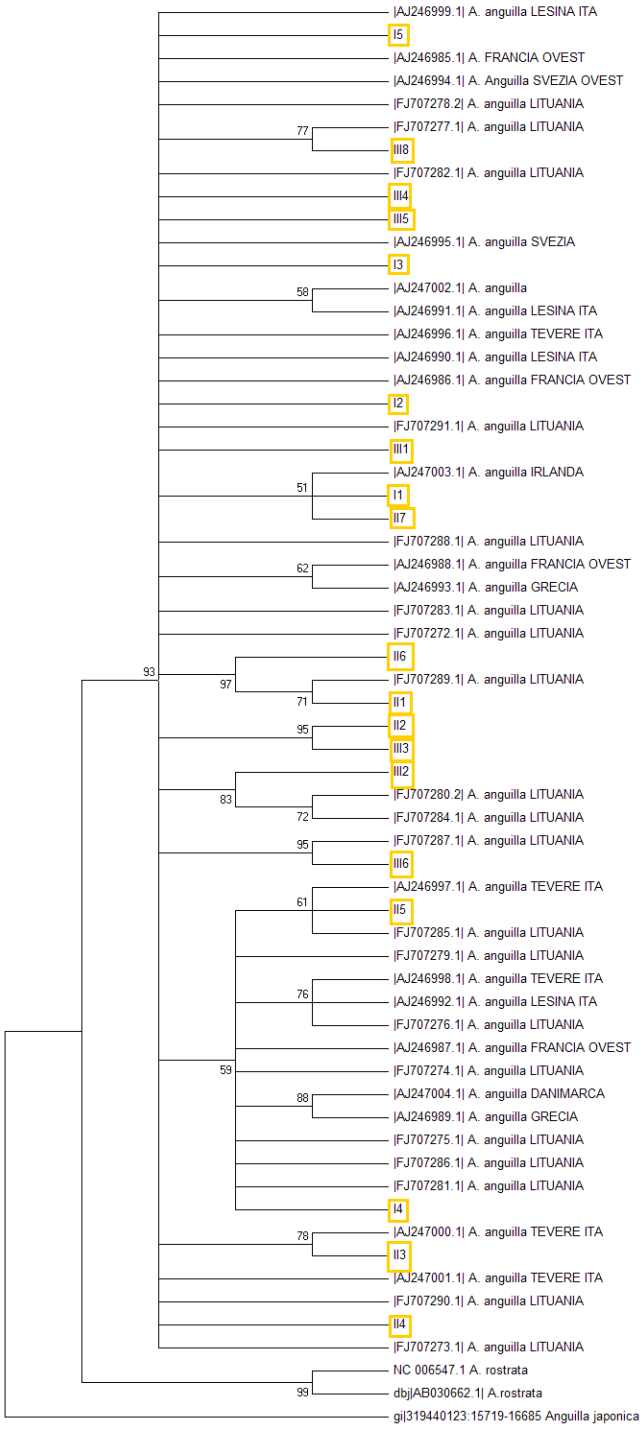


Figure 4.7.1. Phylogenetic tree for silver eels captured in the Comacchio lagoon (N=19).

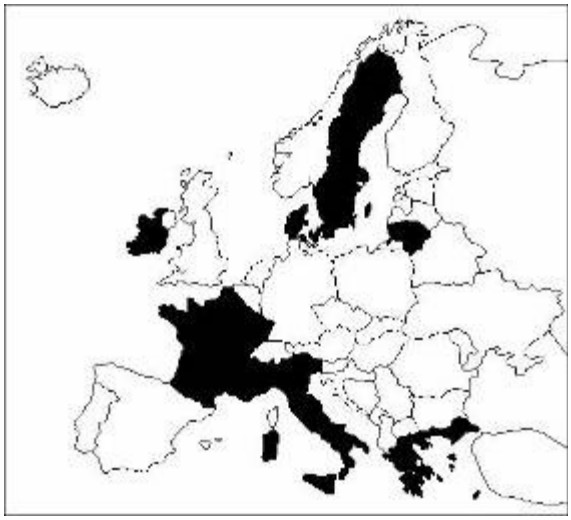


Figure 4.7.2. Maps of European Countries and capture areas (black) of eels near to samples from Comacchio lagoon eels in phylogenetic tree.

5. Discussions

Sophia Loren in the movie La donna del fiume (1955). On the background, the cans used in the Italian market, while Sofia holds the squared one delivered to the U.S.A..

5.1 Estimates on eels – Group A

Eel captured during 2006-2010 in the Comacchio lagoon showed consistent average length and weight estimates of about 800 mm and 1200 g, respectively. A large frequency of lower classes for both total length and total body weight was recorded in 2009 (Figs. 4.1.1 and 4.1.2); in this year both Condition Factor and Eye Index distributions had entries in the lower frequency classes (figure 4.1.3 and figure 4.1.4). Such changes may partially be explained by a larger use of fyke-nets instead of the *lavoriero* during the 2009 fishery season. This hypothesis is supported by the Silver Index (SI) distribution (figure 4.1.5 and graphically discussed in figure 5.1.1), where the first stages of silvering (stage 1 and 2) were present. Silvering is a gradual process that for female eels includes five stages: resident (no gonadal development or I; with visible ovaries or II), pre-migrant (beginning of gonadotropin synthesis or III), and migrant (first downstream movements or IV; actively migrating or V) eels (EELREP, 2005). In contrast, when eels were harvested only using the lavoriero, the first two stages were totally absent (figure 4.1.5 and graphically discussed in figure 5.1.1).

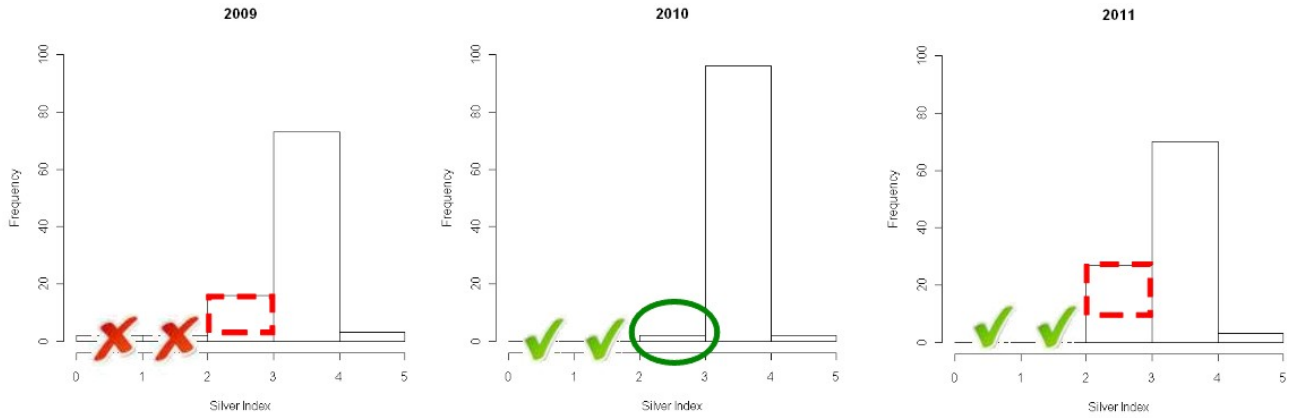


Figure 5.1.1. Silver Index frequency distributions in Group A.

The SI is used to evaluate sexual maturity of large numbers of fish without sacrificing the animals. Although the SI can be affected by error (EELREP, 2005), we argue that a sustainable eel fishery should provide a SI distribution similar to those found in 2007 and 2010. Likewise, the Eye Index in 2007 and 2010 shows the highest values, with only a few specimens under 6.50 (2% in 2007, 0% in 2010). An EI value of 6.50 is presently used as the cut-off value for yellow to silver eel transition, in agreement with other authors (van Ginnecken et al., 2007). For silver eels captured in the Comacchio lagoon by the lavoriero, Colombo et al. (1984) reported total body length at sexual differentiation was 250-330 mm for males and 300-450 mm for females. Similar differences were reported by Carrieri et al.

(1992) who showed a maximum length of 465 mm for males and a minimum length of 525 mm for females at the migrating stage. The sex of eels classified in the present study as Group A was assumed based on total body length. Group A eels were predominantly females (96.86%; 3.14% of the 2009 specimens were undetermined). Because of the low productivity of the Comacchio lagoon (<0.1 ton/km^2), a low density population is presumed to exist here. The morphological estimates support the hypothesis that eels growing at low density tend to differentiate as females (Tesch, 2003; Davey and Jellyman, 2005). Nevertheless, further possible influences on growth rate including food web and anthropogenic disruptions (e.g. endocrine disruptor releases into the basins) should be investigated. A first study on pesticides exposure of silver eel caught in the Comacchio lagoon was recently reported by our laboratory (Valbonesi et al., 2011). Silver eels are sensitive to pesticides, however acethylcholinesterase activities evaluated in tissues of specimens from the Comacchio lagoon were high and consistent with good quality waters in the lagoon. Group A shows eels captured at the lavoriero, in the lagoonal channels that link internal waters and open sea. Here only animals moving spontaneously are captured. The same result cannot be achieved with fyke-nets, located elsewhere in the lagoons. The fishery with lavoriero can be defined as “sustainable”, because the fishery effort is performed only on mature eels, with the biggest weight. This means a reasonable use of eels, in food processing, natural restocking (release of silver eels into the sea) and artificial reproduction.

5.2 Estimates on eels – Group B

Group B eels consisted of 100% females are proved by all indices, such as SI, EI and ASR. The GSI assessed was near (2010) or greater (2009, 2011) than 1.50, which corresponds to mean for mature individuals according to EELREP (2005). This result supports the appropriateness of use of external indices for evaluation of eels. Haematological parameters (RBC=$1.7 \cdot 10^6$ cell/mm^3, WBC=$60 \cdot 10^3$ cell/mm^3, HT=33%) are consistent with previous reports (Tesch, 2003) and can be taken as reference values for silver eels harvested in the Comacchio lagoon. Use of MS222 or newest products for anaesthesia (admitted for human use) should be investigated, and the haematological values may be considered as indicators of change in fish biology.

Stage	Condition	Behaviour/Response
I	Sedation	Motion and breathing reduced
II	Anaesthesia	Partial loss of equilibrium. Reactive to touch stimuli
III	Surgical anaesthesia	Total loss of equilibrium. No reaction to touch stimuli

Table 5.2.1 Stages of anaesthesia in fish.

During preliminary test (Spring 2011), Fenoxy (produced by Oxigen) was evaluated in yellow eels: stage II was achieved in 2-3 minutes and stage III in 5 minutes. The eel population of Comacchio is free from one of the main threats of extinction, as the nematode parasite *A. crassus* was not detected (0%, in agreement with previous studies by Dezfuli et al., 2009). This differs significantly from other European systems (UK, >99%, Gollock et al., 2004; The Netherlands, up to 90%; Haenen et al., 2010; Turkey, 72-82%, Genc et al., 2003). The nematode *A. crassus* was introduced by fish mongers into Europe from Asia (Bruslè, 1994) and rapidly invaded most European water ways. It is located to the swim bladder and it hematophageous diet reduces the oxygen availability and the swimming capacity of the eel. Haenen et al. (2010) states that *A. crassus* is a chronic stress factor for the eel. It interferes with the success of the transoceanic migration of spawners, thus an additional possible cause of stock depletion (Feunteun, 2002). *A. crassus* may cause mortality in wild and farmed eels in association with additional stressors (Kirk, 2003). From this perspective, silver eels from the Comacchio lagoon must be considered in a good health status. Otoliths are small particles, composed of a combination of a gelatinous matrix and calcium carbonate in the viscous fluid of the saccule and utricle of the inner ear. Fish otoliths accrete layers of calcium carbonate and gelatinous matrix throughout their lives. The accretion rate varies with growth of the fish,

resulting in the appearance of rings. Although age determination in eels has been discussed in the past (Poole et al., 2004), counting otolith rings effectively establishes the age of the fish in years. Age can be a significant risk factor in assessing/evaluating the effects of pollution exposure on fish (Myers, 1998) and is an important parameter for planning and performing the EMP. The average age of Group B eels was 9.2±0.5, 9.0±0.3 and 8.4±0.3 years old in the 2009, 2010 and 2011, respectively. Therefore, the group was a highly homogeneous sample consisting of migrating eels of about 1350 g in weight and 850 mm in length. It is worth noting that the size and the 4th silver stage were reached at a relatively young age, of about 9 years (8.8±0.2 as average in the three years). Indeed, Tesch (2003) reported that migrant eels vary from 4 to 6 years and about 400-600 mm in length, to as much as 20 years and about 1000 mm in length. Samples in the Group B show valuable features, and thus are proposed both for natural restocking (release in open sea) and for artificial reproduction (i.e. reproduction induced under controlled conditions). In particular, the methodology used for Group B sampling is proposed for selection of best animals for artificial reproduction trials.

5.3 SEELF

The evaluation of SEELF (both A and B version) can be performed in *classes*, top (from 8 to 10, green in fig. 5.3.1), middle (from 6 to 8, yellow in fig. 5.3.1) and low (from 4 to 6, red in fig. 5.3.1). Another class, from 0 to 4 (white background), can be defined as *unacceptable*.

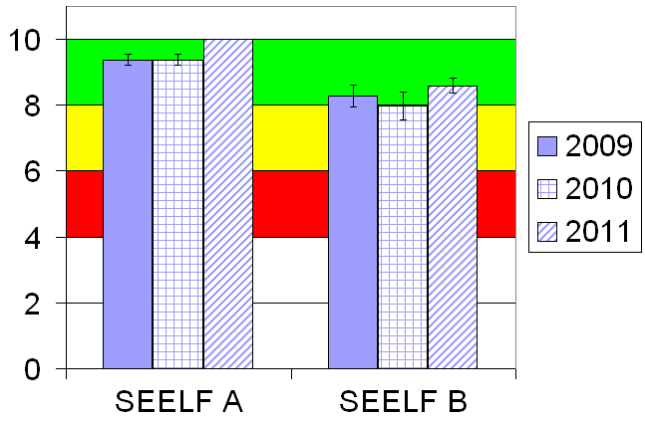


Figure 5.3.1. Comparison between SEELF A and SEELF B in Group B, with quality classes displayed (in SEELF A 2011, SE=0.00).

SEELF A provides a quick evaluation of fish condition and can be used in field, while SEELF B is a useful tool for a more detailed evaluation of fish condition, suitable for research and quality control for food productions. The low SEELF A values recorded in 2008 and 2009 are probably due to inappropriate fishery management, i.e. the use of fyke-nets instead of an exclusive use of the *lavoriero* (Po Delta Park, personal communication). SEELF B showed lower values with respect to SEELF A, indeed, SEELF B provides a more accurate evaluation of fish condition, therefore such a difference was easily predictable. The 2011 provide the highest scores for both indices, with SEELF B of the 2010 is near to the *middle class* and thus, it provides an early warning on fish quality.

The different scenarios for SEELF B calculation, provide a wide range of setting; the best "fit" with information provided by GSI seems to be the scenarios no.6. But this includes a low Condition Factor value (a little more than minimum level for healthy fish), a very large Hematocrit range, although the RBC value is according to Ghittino ($1.4*10^6$ cell/mm^3, for farmed eels, 1983). Eye Index seems a mismatching with SEELF B and GSI (2010>2009); but EI provides an evaluation on external parameters and this could be the reason. Anyway, EI doesn't measure the silvering stage or sexual maturity, but gives an information on the progress of metamorphosis. The elevated value of body indices in 2011 are referred to a sampling provided for evaluation of the biggest specimen, as a requirement for assays on metabolism, performed by EPB Laboratory during Autumn

2011. SEELF seems to provide an adequate description of samples, defining a methodology for fish evaluation. Although modification are possible, the SEELF approach aims to evaluate eel stock and to give a trend in mid-long term. That is reliable and shown in this work. The Comacchio lagoon is historically recognized as an important habitat for eels, while other coastal lagoons, surrounding Comacchio or in other Adriatic coast zone, give different result. The following figure show the SEELF A calculation on Group A and other historical data:

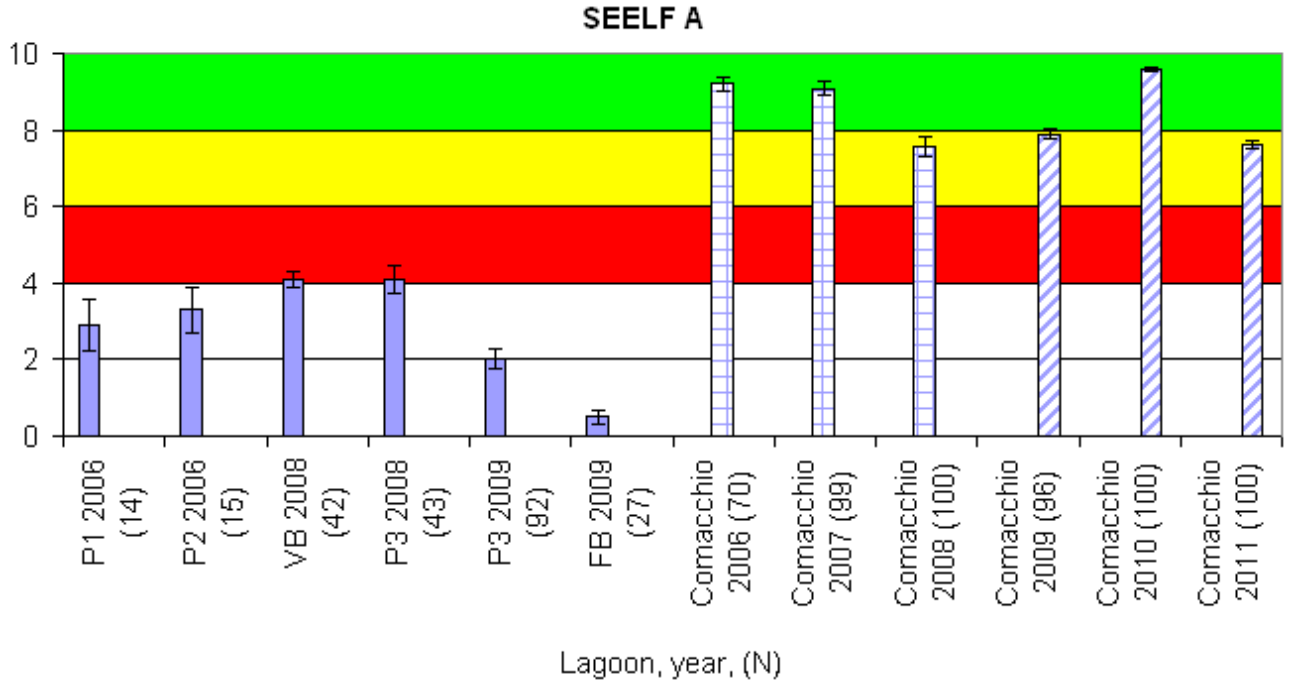


Figure 5.3.2. Graphs of SEELF A calculated on Group A, Comacchio lagoon from 2006 to 2008 and other coastal lagoons (from 2006 to 2009), with quality classes displayed. Data are reported as mean±SE.

As reported in figure 4.3.2. and discussed in figure 5.3.2., other coastal lagoons provide stock with less interesting features, because of a low value of SEELF A, classified in *unacceptable* class. Although evaluated in few samples (from 14 to 92), these lagoons are very far from the performance measured in the Comacchio lagoon. The variability in Group A score is explainable by the use of fyke-nets during the eels captures, as confirmed by the Po Delta Park (personal communications). During one or more harvest, the personnel did use the fyke-nets and this was easily evaluated during the morphometric estimates. However, there is no an official registration of period of capture-tools of capture, and this discussion is still an hypothesis, even if informally confirmed. A similar reason is addressed to P3 scores in 2008 (4.1) and 2009 (2), but no official details on capture tools are available. Both differences in Comacchio and P3 lagoons show that management capability are dramatically important for sustainable eel fishery.

5.4 Calibration between otoliths and fish scales readings

The age of eels at the time of migration, and so the time needed for eel to reach sexual maturity, is of interest for both fish farming and for eel conservation. Age determination of eel was discussed by Poole et al. (2004), and can be affected by errors due to sampling, preparation and reading. It can be a significant risk factor in assessing and evaluating the effects of pollution exposure on fish (Myers, 1998). Despite these limits, the age estimation of a large number of samples is a useful information for planning and performing of the Eel Management Plan (EC, 2007). The present work provide a calibration between eel age by otolith and scale reading, between the two methods, based on scale reading as a quick and non-destructive method for age determination. The variability in Δ factor can be due to otolith preparation, age reading (both in otoliths and fish scales) and, as reported by Tesch (2003), due to variability in scale growth. For this topic, is necessary to improve the number of specimens as well as to continue to select a narrow homogeneous samples such as female at 4th stage of silvering. Otolithometry was performed with an improved technique, using a new device (OP - otolith polisher). All samples were fully readable. With other techniques, otoliths are often damaged during experiments and the otolith reading in *Anguilla* sp. can be affected by a percentage of unreadable or ambiguous otoliths that ranges from 3 to 44% (Graynoth, 1999). The use of the new low-cost otolith polisher, described in 3.3.1, allows investigations on the age of *A. anguilla* using a minimum number of animals, which is of course important for evaluation of an endangered species. Average age of eels collected at the onset of their reproductive migration from the Comacchio lagoon, according to the otolith reading, was 8.8±0.20 (mean±SE) years. This result is in agreement with previous estimates on the same population performed by Rossi et al (1988), in this lagoon. The age evaluated in mature eels collected along different European sea coasts was instead much higher, up to 20 years (Tesch, 2003). Age determination of the 22 eels, through fish scalea reading, was about 6.8±0.2 (mean±SE) years. This difference was consistent between the two methods. A factor (Δ) for the calculation of age from fish scale readings to otolith readings, and *vice versa*, was developed; the linear relationship between the two set of measurements confirms that a calibration otolith-scale is meaningful on this eel stock. At the same time, a calibration between destructive and non-destructive methods is appropriate in the management of endangered species as the European eel. Tesch (2003) discussed the issue of age determination and age at maturation of eels, reporting great variability due to different methodologies of eel capture and age measurement, due to difference in the living

environment. We consider that the application of the proposed methodology should require preliminary studies when applied to eels collected from different habitats.

Further comparisons between otoliths and fish scales were recently performed by mark-recapture experiments in different fish species (Zymonas and McMahon, 2009; Schill et al., 2010). These validated annulus periodicity, rather than absolute age, but the Δ factor was not evaluated. Zymonas and McMahon (2009) compared pelvic fin rays, scales, and otoliths of bull trouts, finding that ageing precision was greater with fin rays and otoliths, than fish scales. Schill et al (2010) performed a 1 year study on redband trouts, also finding scales less precise than otoliths. Although its limitation (i.e., it does not provide absolute age), the mark-recapture methodology is useful for the evaluation of growth of *A. anguilla*, and a mark-recapture trial is planned to improve of current estimates.

5.5 ChE assays

5.5.1 ChE forms characterisation

Although not solely responsible for eel population declines, chemical pollution could well be a substantial contributory agent to reduce the health status of spawners, and may also limit recovery from other causes of stress. Recent reports provided strong indications for the destructive effects of water pollutants in eel, concluding that in areas with high levels of contaminants accumulating in adipose tissue, even those eels that have very high scores for the reproduction capacity cannot participate in successful production of vital offspring (Van Ginneken et al., 2009). Due to the possible relationship between pesticides and eel energetic and health status, the study on ChE activity and sensitivity to pesticides in silver eels at the onset of the reproductive migration is a priority. ChE are highly polymorphic enzymes (Bebianno et al., 2004) present in different forms, i.e. acetylcholinesterase (AChE) and pseudocholinesterases, butyrylcholinesterase (BChE) and propionyl-cholinesterase (PChE). The physiological role of AChE is widely known, leading to the acetylcholine breakdown in cholinergic synapses. The role of BChE and PChE is less understood due to the lack of a natural substrate; nevertheless, they are present in several tissues including blood, and seem to be involved in detoxification processes, cell regeneration, etc. (Mack and Robitzki, 2000). ChE activities were analysed in four silver eel tissues in the presence of different substrates. Hydrolysis of ASCh was clearly the predominant one in all tissues analysed. A smaller but significant percentage was represented by BSCh hydrolysis in the liver and plasma, probably related to its non-neural activities. A wide difference in total ChE activities amongst tissues was observed: brain and white skeletal muscle showed similar activities, which were about 5-fold and 10-fold higher than those measured in liver and plasma, respectively. This finding is in agreement with previous reports (Gant et al., 1984) which indicated that in most fish ChE activities in liver, kidney and plasma are lower than in other tissues. Plasma was previously reported as the tissue showing the highest degree of AChE inhibition after animal exposure to pesticides (Thompson et al., 1991; Cerón et al., 1996). As a matter of fact, human plasma AChE is used to monitor exceeding exposure of workers dealing with pesticides (Ballantyne and Salem, 2006, Remor et al., 2009). In this work, ChE activities were evaluated in plasma samples to possibly develop a non-destructive assay for detection of eel exposure to pesticides. Besides the difficulties to obtain the blood from living eels, as well as to collect unclotted blood samples from dead animals, our findings indicate that plasma ChE activity is rather low, posing some limitations when searching for its inhibition.

Therefore, inhibition of ChE activity in brain and skeletal muscle appeared the best candidate as a biomarker of pesticide exposure in silver eel, and further experiments more deeply investigated ASCh hydrolysis in these tissues.

5.5.1.1. Characterization of cholinesterases

Evaluation of ChE kinetic parameters provided similar Km (0.31 and 0.30 mM), and Vmax (40.28 and 35.47 nmol min^{-1} mg $protein^{-1}$) values for brain and white skeletal muscle, respectively. Within the same tissue, a very small variation was detected among individuals in terms of enzyme activities. This appears related to the highly homogeneous experimental sample used in this study. Indeed, fish ChE activities is modulated by seasonality, habitat, food chain and biological parameters including age, sex and size (Gold-Bouchot et al., 2006; Solé et al., 2008, 2010; Varó et al., 2008). Significant variations in ChE activities and predominance of protein isoforms were reported in several fish (Solé et al., 2010). Features of our sample were compared with previous reports dealing with ChE activities in *A. anguilla* (Table 4.5.4). It is worth noting that all studies analyzed eels at the yellow life-stage, with the exception of the present one; moreover, animals of significant smaller size and maintained in tap or sea-water were investigated, while specimens from the Comacchio lagoon were much larger in size and grown in a brackish coastal lagoon. Sex was mainly undetermined, while our sample was composed entirely of females. More specifically, they were silver eels at the fourth stage of development. Despite these differences, silver eel brain ChE specific activities were quite similar to those in yellow eel reported by Gravato et al. (2010) and Guimarães et al. (2009). Not much data are available for muscle, liver and plasma and a true comparison cannot be made.

5.5.1.2. Use of inhibitors to identify ChE forms

Eserine is a non selective ChE inhibitor and, as expected, it greatly inhibited brain and muscle ChE activities in a dose-dependent manner, with a maximum effect at 10^{-5} M; at this concentration ChE hydrolysis was reduced more than 90% with respect to basal values. Since our protein preparation was poorly purified, a number of non-specific esterases able to metabolize ASCh might have been present. However, the high sensitivity to eserine confirms that non-specific esterases represent a very low component of ChE in silver eel tissues. Selective inhibitors were used to distinguish among different ChE forms in fish (Chuiko et al., 2003; Alpuche-Gual and Gold-Bouchot, 2008; Solé et al., 2008) and other vertebrates (Sanchez-Hernandez and Moreno-Sanchez, 2002; Kousba et al., 2003).

The significant enzyme inhibition caused by BW 284c51 (AChE selective inhibitor) and the lack of effect by iso-OMPA (BChE selective inhibitor) indicated that AChE was the predominant ChE form in the eel tissues analyzed.

5.5.1.3. In vitro ChE inhibition by pesticides

The effects of pesticides on ChE is not novel, and they were well documented in yellow eel after in vivo exposure. Eel exposure to 11.15 mg/L molinate (up to 96 h) caused a maximum inhibition of about 70% and 19% of plasma and brain AChE activities, respectively (Sancho et al., 2000a). Thiobencarb (0.22 mg/L; 2–96 h) reduced AChE activity in eel eyes up to 75% (Sancho et al., 2000b); at the same water concentration, the pesticide reduced AChE activity in muscle of about 70% after 2 h (Fernández-Vega et al., 2002). Fenitrothion inhibited about 60% enzyme activity in brain (Sancho et al., 1998) and plasma (Sancho et al., 1998) at a sublethal concentration of 0.04 mg/L (96 h). Yellow eel exposure to diazinon (0.042 mg/L up to 96 h) caused a reduction of about 79%, 99% and 85% in brain, plasma and eye ChE activity, respectively (Cerón et al., 1996).

Data on in vitro effects of pesticides are not available for eels. Present data on silver eels show that ChE activities in brain and muscle were significantly affected by *in vitro* exposure to pesticides. Carbamates had stronger effects, with IC50 slightly lower in muscle with respect to brain, whilst organophosphates were weaker inhibitors of eel ChE. As shown in Table 4.5.3, present data are in agreement with those from previous in vitro studies. It is worth noting that acute organophosphate toxicity is strongly augmented by bioactivation processes mediated by Cyt P-450 in living cells (Livingstone, 2000), thus pesticides of this class have the potential to cause much stronger effects after in vivo exposures.

The ChE inhibition by pesticides is important for an evaluation of ChE form and its physiological role, with particular regards of detection of environmental pollution. The in vitro exposure to pesticides was detected both in brain and muscle; thus, the use of muscle as target for future ChE assays is reasonable both for practical (easy sampling, compatible with food processing) and scientific (detected inhibition) topics.

5.5.2 AChE assays

The AChE was evaluated in several targets, organs (brain, liver), muscle and plasma. Some requirements were used for selection of appropriated target, i.e. 1) easy sampling and 2) high value of measurement. In fact, the sample should be taken from a carcass used into the food processing and the value should be reliable high to be compared in a long series also within environmental variability and inter-variability. During the food processing of Comacchio's typical product, the animal are sacrificed by fast decapitation and muscle sample are easily available. The measurements of AChE on skeletal muscle is properly applicable for eels characterisation and for environmental monitoring, as discussed in 5.5.1. The estimates on 3 years samples (N=24) provide a reference value of 22.7±1.99 μmol min^{-1} g $protein^{-1}$. This result was obtained without anaesthesia, on sample captured with the lavoriero. Although in according to Valbonesi et al (2011), further investigation should be performed on a wider sample, also with evaluation on possible effects due to anaesthesia. This work is focused on the characterisation of silver eels and AChE reference value is an important milestone for monitoring of eels health status. Although few historical data are available, this parameter is proposed as a measurement on biological indicator (eel) for a qualitative evaluation of environmental quality (coastal lagoon). Therefore, the eel is proposed as biological indicator for environmental monitoring.

5.6 Blood biochemistry

Because of a standard biochemical profile is not available for fish, in these experiments we basically used the profile for mammals (human included): glucose, urea, aspartate amino transferase (AST) (EC 2.6.1.1), alanine aminotransferase (ALT) (EC 2.6.1.2), alkaline phosphatase (ALP) (EC 3.1.3.1), lactic dehydrogenase (EC 1.1.1.27), creatine kinase (EC 2.7.3.2), Ca, P, Mg, Na, K, Cl, total protein, albumin, triglycerides e total cholesterol.

These experiments provide the very first data on bloom biochemistry for *A. anguilla*, so these are original results, useful both as reference for the species and for the stock of the Comacchio lagoon. The results are in according to other measured in teleostei (Folmar, 1993), and they can be described as good quality indicators. The 2009 did provide some useful indications for improvement of sampling, because of some stress-related parameters (e.g. capture and transport) show elevated values: glucose, ALT, AST, Ca, Na e albumin. The high values of glycaemia and activity of enzyme (as LDH, CK, AST and ALT), can be due to stress for the transport protocol in 2009. Stress due to sampling can give iperactivity to fish, damage the muscle and release enzyme as CK and LDH, as reported for *Cyprinus carpio* (Palmeiro et al., 2007). Furthermore, blood serum enzymes shows a bigger inter-variability than other blood chemistry parameters (Folmar, 1993; Shahsavani et al., 2010). From 2010, the transport protocol was improved and thus estimates show better results. Blood biochemistry evidences can be influenced by environmental condition, such as long-term exposure to pollution (Folmar, 1993). Different results from sampling could be due to physiological response to exposure to pollution. Although closed to the sea and to freshwater channels, the Comacchio lagoon can be affected by pollution during brief inlet of freshwater (few days per year – this action is performed by the Po Delta Park, during maintenance of pomps located in the Southern boarder of the lagoon). This can collect nutrient load as well as chemicals produced by agriculture, in a wide land reclamation area located in the surrounding of the lagoon. For example, in *Lepomis macrochirus*, an increase of glycaemia was associated to exposure to Cu (Heath et al., 1991). Furthermore triglycerides e total cholesterol are higher than value detected in *Acipenser brevirostrum* (42÷133 mg/dl; Knowles et al., 2006), in tilapia (178 mg/dl; Chen et al., 2004). Higher values are reported in salmonids (Folmar 1993); in *Oncorhynchus mykiss,* Manera & Britti (2006) give cholesterol 247mg/dl and triglycerides 347mg/dl. The measured cholesterol and triglycerides could be physiological reference for silver eels, rich in lipids, in the right condition for the reproductive migration (Lintas et al., 1998).

5.7 Genetic investigation

The samples from Comacchio lagoon show an high inter-variability, as reported in Figure 4.7.1. The animal are distributed on several branches of the phulogenetic tree and this proves that the Comacchio's stock is not isolated and there no a bijection relationship between juveniles, spawner and breeding area. This population is not closed and the natural restocking is provided by specimens that origins from spawner of all continent (Barbara Rossi, 2010). Comacchio's samples are similar to animals from Denmark, France, Greece, Ireland, Lithuania, Sweden and other Italian sites as a wide distribution over the whole Europe. Silver eels escaping from the Italian coastal lagoons can provide the juveniles for other areas and *vice versa*. But natural restocking from other European habitats is not evaluated, and contribution to natural restocking of European eel population cannot be discussed. The experimental evidences here provided agree with panmixia theory, as previously discussed by other authors (Palm et al., 2009; Pujolar et al., 2011; Als et al., 2011), and suggest that Comacchio lagoon, as well as other Mediterranean coastal lagoons, can provide reliable spawners for eel restoration in whole Europe.

6. Conclusions

Sustainable eel fishery means of a cultural revolution.

6.1 Estimates on eels – Group A

Eels captured by lavoriero and randomly selected, show consistent average in total length, total weight, Condition Factor and Eye Index and the Silver Index of migrating eels. All estimates are good values for silver female eels and this proves that the Comacchio extensive breeding area provides female specimen with a huge growth rate. The use of the *lavoriero* assures capture of only mature migrating females (both by Eye Index and Silver Index). The use of external indices for fish evaluation has been tested for Comacchio lagoon and, thus, in the next future eels can be used for quick health status monitoring by field research and catch&release methodologies. A fish evaluation on the basis of external parameters is important for the application of the Eel Management Plan, both in coastal lagoon and in riverine waters. A comparison of Group A parameters of eel stocks from different areas and environments can help in breeding areas analysis and can improve the knowledge on their potential for eel conservation. Furthermore, the eel conservation should be performed according with fishermen and local communities, because of a historical presence on the water bodies and ability to identify natural habitats for eels.

6.2 Estimates on eels – Group B

Eels captured by *lavoriero* and properly selected for evaluation of spawners and natural restocking purpose, were evaluated in a wide range of body indices (external and internal), blood parameters and age. All parameters were better that Group A (captured in the same period), because of a more mature condition, as proved by internal indices (gonado-somatic index is the most important). This supports the appropriateness of use of external indices such as SI, EI and ASR in selecting mature eels. Age of this Group can be considered as the age at maturation in internal waters in the Comacchio lagoon and this is a key-factor for eel conservation and EMP. In fact, the effectiveness of EMP is dramatically influenced by the age at maturation, as well as youngest mature eels are preferable. Age by otolith reading and fish scale reading are both suitable, but a calibration has to be performed before using the non-destructive method. This was proved for an homogeneous sample of silver eels and use on eel stock (including all size) is not proved (see 6.4). As results, reference for future measurements and basis for further evaluation on local eel stock and spawners are provided. In fact, although most of the eel growth in the Comacchio lagoon may be released in open sea, only the biggest should be used in

artificial reproduction trials, because of the best values in all parameters and the largely harvested. Moreover, using the methodology for sampling of Group B (by ASR) the best specimen can be selected for future experiment in physiology, reproduction and other topics. The capability to select the best sample available in an environment, should be considered as an important task for every Organisation in charge of the management of habitat and biodiversity conservation. Surely the Comacchio lagoon is a perfect study-case, but similar experience should be exported to other sites in Europe. Regarding the EMP, this methodology should be used for testing of the release of eels in open waters, monitoring of the health status of escaping fishes and monitoring of the age at maturation, as priority of future EMP.

6.3 SEELF

The SEELF index aims to provide a methodology for measurement of eel conditions, according to use of fish: aquaculture and other commercial uses (SEELF A) and quality control and research (SEELF B). Both indices were designed as a practical solution for a very complex problem, as well as the evaluation of a specie during a metamorphosis is a huge scientific task. Although some limitations in the use of indices, the Eel Management Plan needs of some tools for fish evaluation, for analysis of trends, both in the same habitat and between different environments. SEELF A and B were designed for silver eels and not exclusively for specimen from the Comacchio lagoon, even if the scores of Comacchio seem to be very high. Application of SEELF methodology and indices to other lagoons was also performed and the importance of a proper water and fishery management was highlighted. The use of classes for evaluation of SEELF score aims to help managers to select the more appropriated habitat for eel stocking: top class (score from 8 to 10) defines areas with good performance, when further efforts can support more effectively the EMP; the middle class (from 6 to 8) defines area with good a potential, but where EMP should be carefully evaluated, and these areas should play the role of buffer areas; low class (from 4 to 6) defines a class of habitats where the environment should be improved for the eel conservation and/or where the targets of environmental management should be more addressed to eel conservation. Values below than 4 are provided by habitats with no management tools (e.g. open lagoons), where no practical action can assure a more suitable environment for eels. More in general, the designation of restocking area and other area where to spend public funds for restoration of habitats for eels, should include a methodology as the one proposed by SEELF.

6.4 Calibration between otoliths and fish scales readings

Lethality of fish otolith sampling poses an important consideration for threatened species such as *A. anguilla*, and a methodology for stock monitoring and management avoiding the sacrifice of animals is a key factor for eel management. Otolith and scale readings were compared for the development of a non-destructive methodology for age determination of European eel breeded in a the Comacchio brackish coastal lagoon. As a result, a conversion factor (Δ) was defined for calculation of otolith reading from scale reading and *vice versa*. The Δ factor (Δ=2) here provided, obtained evaluating an highly homogeneous sample of 22 female silver eels, is in agreement with Tesch (2003). As an overall, present data point out the young age (about 9 years) of female silver eels breeded in the Comacchio lagoon at the onset of their reproductive migration, in comparison with that evaluated in eel from other European environments (Tesch, 2003). These experimental evidences suggest that the Comacchio lagoon is a reliable site where the application of the Eel Management Plan can be effective.

6.5 ChE assays

6.5.1 ChE forms characterisation

Many physiological changes deserve explanation and need comparison between silver and yellow eels, so the present contribution represents only a small part of future expected elucidations. ChE activities in silver eels show a tissue distribution similar to that observed in other teleosts, with some exceptions mainly related to the extent of BChE activity in plasma (Solé et al., 2008, 2010). The relatively low plasma ChE activity and the difficulties to obtain blood samples from living eels seem to limit the possibility to use plasma for a non-destructive assessment of ChE activities in these animals. As to pesticide effects, the inhibition of silver eel ChE was in the range of that measured in other fish. Nevertheless, given the peculiar life-cycle of eels, pesticides could hamper the process of energy storage during the yellow phase and increase the consumption of energy reserves useful for migration and reproduction. Moreover, pesticides may impair the delicate swim efficiency/energy balance during the migration phase since ChE inhibition is particularly relevant when pesticides are mobilized together with fat consumption during prolonged swimming (van Ginneken et al., 2009). Considering that pesticides tend to undergo rapid degradation in water, while their effects on animals remain for several weeks, a strategy involving the inhibition of ChE activity as a biomarker is a suitable alternative for assessing the animal exposure to pesticides (Viarengo et al., 2007). According to the present data,

ChE inhibition can be used as a biomarker for pesticide exposure in silver eel, as it was in yellow eel (e.g. Guimarães et al., 2009) and other teleost fish (e.g. Hernández-Moreno et al., 2010). This would contribute to the monitoring of environmental health as well as of the quality of spawners before they leave the continental waters, being crucial for understanding whether the quality of the continental habitats guarantees the physiological completion of the life-stage. The concern for eel health status parallels a theoretical risk to humans who consume these fish; in fact eels represent a valuable commercial inland fishery in many countries, including the area of Comacchio, Italy (Tesch, 2003). Therefore, determination of ChE inhibition as an early warning index of pesticide exposure would provide an important support to management actions undertaken in continental waters, and to ensure food quality.

6.5.2 AChE assays

AChE in organs, tissue and plasma of eels are usable target for AChE estimates, and measurement on skeletal muscle was designed as reference for silver eels. Muscle is easily sampled in eels used in food processing; the availability of a factory for production of marinated eel in Comacchio is important in order to have a wide sampling in the future. Using a biomarker as ChE measurement, is possible to have information on dispersion of chemicals into the water and to identify sources of contaminations. As stated by Valbonesi et al (2011), the eels breeded in the Comacchio lagoon can be considered as healthy animals, and future experiment can provide further information for a long-term evaluation.

6.6 Blood biochemistry

Blood features, as counts (RBC and WBC) , HT and biochemistry, can be performed on juveniles, yellow and silver eels, sometimes without sacrificing the animals. Glycaemia and some enzyme (such as LDH, CK, AST and ALT), should be considered as stress-correlated indicators and estimated as response to stress at capture and transport. Here measured in silver eels, these parameters should be evaluated also in yellow eels, in order to provide an information on all stages of eel's biological cycle. References values are here provided and future estimates will be compared with these first results.

6.7 Genetic investigation

A phylogenetic tree is a diagram showing the relationships between specimen upon similarities and differences in their genetic features. The displayed results show clearly differences between the rough 20 samples, confirming the theory of panmixia for the European eel. These important results on eels from the Comacchio lagoon is a substantial part of the proposal of definition of European Restocking Area (ERA – see 6.8.1). ERAs have to provide healthy mature eels for restocking and should be areas where International effort is funded by EU. The panmixia hypothesis is thus very important as well as ERAs are dramatically necessary for a quick recovery of European eel population.

6.8 Effectiveness of EMP performed in Mediterranean coastal lagoon

The Council Regulation (EC) No.1100/2007 *establishing measures for the recovery of the stock of European eel*, defines a long-term target for the release in open sea of silver eels biomass (40%, article 2.4) and states that a Eel Management Plan can involve measures such as (article 2.8):

- reducing commercial fishing activity,
- restricting recreational fishing,
- restocking measures,
- structural measures to make rivers passable and improve river habitats, together with other environmental measures,
- transportation of silver eel from inland waters to waters from which they can escape freely to the Sargasso Sea,
- combating predators,
- temporary switching-off of hydro-electric power turbines,
- measures related to aquaculture.

Although the “40%” is a long-term target and the above list is not complete, some topic are in the dark:

a. How to select the fish to release into the sea ?
b. Where the eel restocking is suitable?
c. How to improve the synergy between aquaculture and conservationism, in order to avoid the collapse of firms and local economies?

Regarding these queries, this thesis provides some practical proposals.

a. How to select the fish to release into the sea ?
The eels released into the sea should be evaluated by quick methods, using external parameters. Based on a ranking, the release should select the best specimens, those with the highest probability to complete the reproductive migration. SEELF is a proposal, and should be implemented or modify (setting of threshold and range used in index calculation) for the use in other environments. SEELF means of Condition Factor, Eye Factor, Silver Index, the most commons indices for evaluation of growth and sexual maturation of eels.

b. Where the eel restocking is suitable?

The restocking should be performed in areas where the age of maturation is smaller, parasite infestations are not present (first *A. crassus*) and the management of basins can be performed properly. The Comacchio brackish lagoon has all requirements and should host a huge effort for restocking. On the other hand, where the age at maturation is >15 years, or where mortality increase due to parasite infestations, the EMP is performed but doesn't make sense; it is only a compliance of a EU regulation, without practical results. In this framework, the opinions of protected area managers, producers and scientific Institutions should be carefully evaluated. The restocking of European eel can be effective only with cooperation between public bodies and private companies. This Public Private Cooperation is running in Comacchio area, even if with not yet acceptable results.

c. How to improve the synergy between aquaculture and conservationism, in order to avoid the collapse of firms and local economies?

In order to solve this question, we should use the eel paradox: to eat, to defend. A restocking following the *business as usual hypothesis* cannot be successful because private cannot economically survive to a business plan with respect of a biological cycle of 10 years or more. The partnership between public and privates should be based on the availability of public fund for restocking (in suitable areas, as discussed in point A) and sustainable eel fishery by privates. Sustainable eel fishery means of respectful production and commerce of an high value product (hatchery) or, when possible, a value-added product. As stated by Steven (2008), a value-added product is a food product that is converted from raw product through processes that give to the resulting product an "incremental value" in the market place. For fishery this kind of process is potentially reliable, also because often eels are breeded in protected areas (e.g. the Natura200 site surrounding Comacchio). As discussed more in details in Annex 2, a sustainable eel fishery can includes a self-financing of the protected area and of biodiversity conservation, by commerce of typical products.

6.8.1 Proposal for an effective EMP

In order to boost the restocking of European eel at continental scale, the following items are proposed:

A. Designation of European Restocking Areas (ERAs)

The ERA is defined as a closed basin, where restocking can be performed effectively, regarding the following requirements:

a. habitat with a good ecological state
b. young age at maturation of eels
c. absence of parasites
d. capability to adapt environment and human practices for a sustainable eel fishery

a. The good ecological state can be monitored both with water quality probe, sediment assays, but also with the eels itself, by bio-accumulation of chemicals and using biomarkers, such as ChE, as discussed in 5.5.2. For this scope can be useful the use of a *mobile laboratory*, as available in the Po river basin (figure 6.8.1, Brunelli 2011).

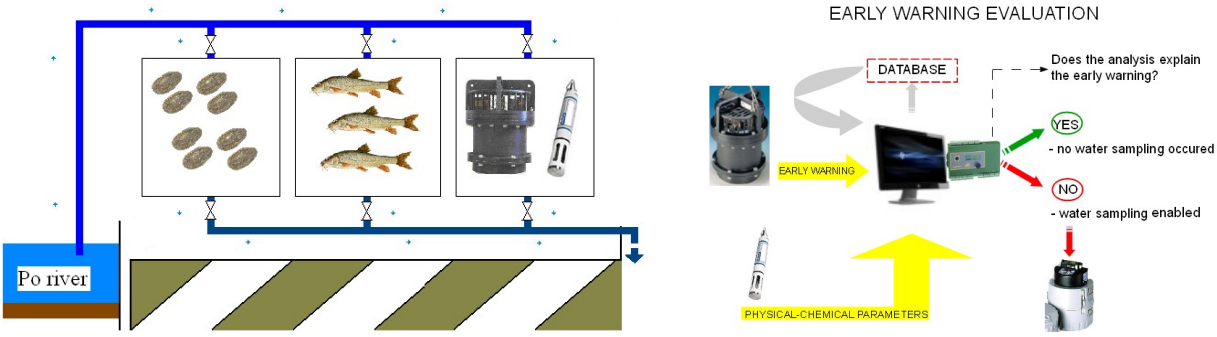


Figure 6.8.1. Scheme of *mobile laboratory* (left) and *early warning evaluation* (right).

The mobile laboratory approach provides a technical solution with some advantages of field sampling (e.g. real time water monitoring) and laboratory set-up (e.g. reduction of environmental variables).

b. The young age at maturation can be evaluated both on alive fish and carcass, with fish scale and otolith readings. In this work a calibration between the two methods was discussed, and a similar approach can be performed in all habitat, with a basic knowledge of stock growth and dynamic (sources of stocking). Because of the fish scale-otolith readings calibration, the age can be estimated during catch&release sampling, giving information for improvement of effectiveness of EMP.

c. Parasite infestations are easily detectable, also on the basis of management measurements (are allochthonous juvenile and/or adults introduced? Are quarantine procedures applied?) and historical data (based on previous record). The Comacchio lagoon is not affected by *A. crassus* and thus is a suitable habitat for restocking.

d. The capability to adapt environment and human practices for sustainable eel fishery includes the capability to create, manage and modify basins, little lagoons and water inlet/outlet facilities, in order to create the best environmental conditions for each stage of eel biological cycle. This also means the reduction of predation by ichthyophagous birds (e.g. cormorans, *Phalacrocorax carbo*, declared as least concern - Bird Life International, 2009) and other fishes. In particular, the conflict between two or many biodiversity elements, like eel and cormorant, should consider the capability of some species to move to other habitat (birds) and the obstacles for other species to move and to defend itself (fishes). Differences in protection status (figure 6.8.2) should also be considered.

Figure 6.8.2. The status of *European eel* (left) and *Phalacrocorax carbo* (right) in the IUCN RedList.

Although widely discussed by protected areas' managers, the topic “conflict in the management of endangered species” is a hot topic that needs effective solutions in short time. Definition of European Restocking Areas (ERAs) should be a priority for the European Commission, in order to improve the effectiveness of EMP and to target the funding in the most suitable areas.

B. Planning an European Scale EMP

This thesis discusses the features of the eel stock of Comacchio lagoon, one of the most famous breeding area in Europe. Although historically important (Tesch, 2003), this stock was affected by a lack in information about health status, age distribution and growth of its eels. As discusses by Feunteun (2002), the main continental reasons of eel decline are presence of migration obstructions, fisheries, parasite infestations and effects of pollution. The Comacchio lagoon is an example of suitable coastal lagoon for eel restocking, where inlet/outlet of water can be easily performed and glass eels and juveniles can be managed with proper solutions. In this kind of habitat, each conservation action can be planned and

performed in order to reduce the time for restoration of stock. Furthermore, a strict mandatory implementation of a river basin EMP, without measurements of results, could have negative consequences, as the waste of EU funding and/or waste of time (e.g. trying to restore population in polluted areas or where the age at maturation is achieved at >15 years). As discussed previously in this thesis, several topic are important for a quick eel restocking: environmental condition, food web, capture tools, evaluation of harvest fish, value-added productions. But the most is the boosting of natural restocking of feral juveniles in suitable habitats. As shown in figure 6.8.3 (source: FAO), the eel biological cycle can be supported, capturing feral glass eels, breeding in controlled environment and release adults in nature. Although this approach in farm is expensive on large scale and cannot have a good pay-back, it is affordable using little coastal lagoons. E.g., in the framework of Comacchio lagoon, the “farm” stage can be performed in little basins, where mortality by predation can be controlled properly and the productivity can achieve acceptable rates.

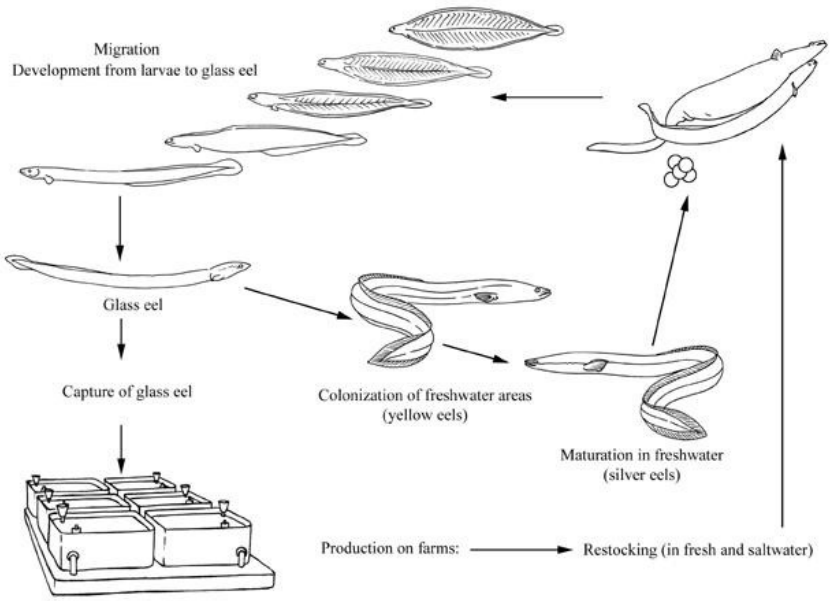


Figure 6.8.3. Production cycle of *Anguilla anguilla* (FAO, www.fao.org).

Trials with feral glass eels, 2 years in age, were performed two consequently times in acquarious by ARPA Ferrara, with survival >90%, during 3 months trial and growth from 0.1 to 1 g in total weight (Emilia-Romagna region Environmental Protection Agency – Ichthyologic Research Unit, Dept of Ferrara – personal communication – figure 6.8.4).

Figure 6.8.4. A glass eel (~2 years old) captured in North Adriatic Sea in 2010 (Courtesy of ARPA Ferrara).

As a concrete proposal, the European restocking effort should be performed only in designed ERAs, assuring reduced mortality, age at maturity <10y and good health conditions (measurable with SEELF index or other indices) of migrating eels.

As a more general proposal, a more effective EMP should consider measures for reduction of eel mortality all over the continent, and should identify ERAs where to use public bodies financial capability in eel restoration. Based on the European scale of the problem (eel decline), the European scale EMP (EsEMP) aims to unify the European Countries in funding eel restocking in suitable areas, ERAs.

Clearly, the EsEMP could be performed only with a large agreement between the Member States and performed by public-private partnership with the most skilled privates.

6.9 Concluding remarks

Different factors are likely to have contributed to eel decline, including overfishing, habitat loss, presence of parasites, climate changes, and poor water quality mainly due to chemical pollution (Feunteun, 2002). The impact of these factors on eel population is exacerbated by the complex biological cycle of the fish, characterized by an extremely long migration in marine waters. Adults die after spawning, while larvae will return to the coastal waters and newly metamorphosed glass eel will migrate upstream to estuaries, rivers, and coastal lagoons. The growth phase stage (yellow eel) in continental waters lasts for several years (6-12 for males and 9-20 for females, Tesch, 2003), and ends with a second metamorphosis called silvering (silver eel), that immediately precedes the transoceanic reproductive migration (Colombo and Grandi, 1995).

Figure 6.9.1. European eel captured in the Comacchio lagoon.

The silver livery of eels is the most apparent external change, used in the past to identify eel at the ‘silver stage’ and implicitly migrants (Pankhurst, 1982). Many other differences have recently been found, including increasing eye size, darkening and length of the pectoral fins that now are commonly used as criteria to determine the life stage of eels (van Ginneken et al., 2007).

Although classified as Critically Endangered by IUCN (Freyhof & Kottelat, 2010), the European eel is still a commercially important species. Nevertheless, nowadays the Eel Management Plan is not designed for an effective restoration of the stock, as well as it doesn’t foresee a cooperative action between areas where the EMP can be performed successfully in short time (5-10 years) and other habitats where it cannot, even if in mid- or long-term (>15 years).

Regulation n.1100/2007 (EC, 2007) imposes on the European Member States the monitoring of the local stocks and the implementation of an Eel Management Plan (EMP) on a river basin scale, for the restoration of eel population. Although the EMP targets can

be considered as long-term objectives, reliable methods have to be developed and applied to make the practical implementation of EMP possible. Moreover, an evaluation of the most appropriate environment for restocking should be performed.

This thesis proposes the use of morphological measurement for evaluation of fish health, in association with internal indices, blood parameters and biomarker (AChE). This is the basis for future research for both biodiversity conservation and eel fishery. Although not widely discusses before by Scientific Community, the need of a SEELF index (or a similar tool) is probably one reason of the ineffectiveness of the Eel Management Plan and, therefore, of the poor condition of most of the eel stocks in Europe. SEELF index is a practical tool and can be used for monitoring of trends, both by the score and by the quality class (top, middle, low, unacceptable). SEELF index can be included in EMP's tools as well as used by protected area managers and farmers for fish monitoring and decision support. This is very important for ERAs managers, that have to improve the effectiveness of each eel growth phase, with particular regarding of mortality due to predators and fish conditions at migration.

The development of a successful and replicable artificial reproduction is even more the "big one" of the eel research. As figure 6.9.2 shows, the knowledge of both eel biological cycle, in nature and culture, is widely known, but some overlapping between known/unknown are present (dotted circles and dashed circle). In this situation, the choice of the priority for driving the scientific effort is dramatically important.

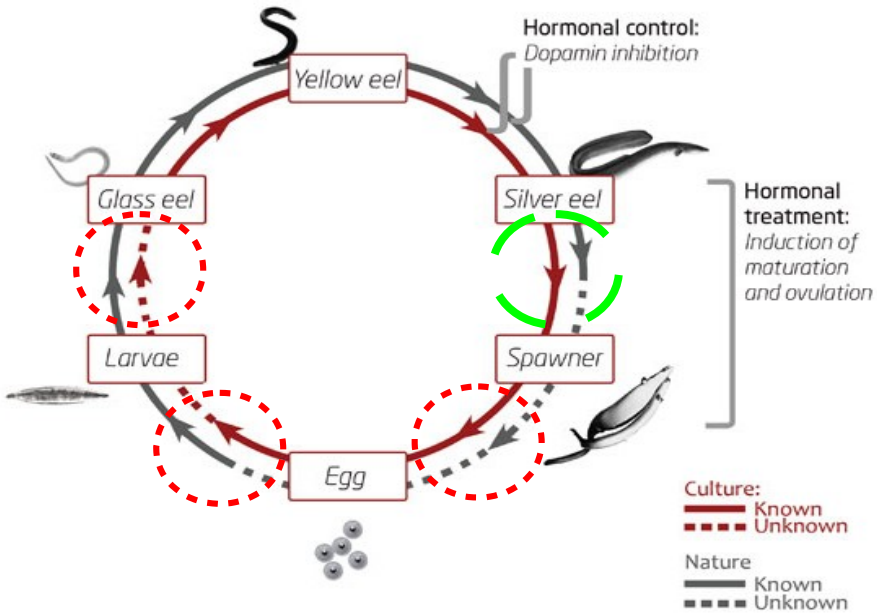


Figure 6.9.2. Knowledge of the biological cycle of European eel, in nature and in culture. (Modified from EELIAD Project).

Between the several options identified by the circles, the first (green) is a full characterisation of the silver eel used for hormonal treatment. This task is possible after a full characterisation of silvering and an evaluation of fish conditions, in order to have a deep knowledge of animals used for reproduction trials (and the capability to sampling such a kind of animals in natural environments). The second topic was widely discussed here, while the first involves a full understanding of metabolism; this is the main research for the EPB Laboratory, where this thesis was performed. This will be a key factor for future improvement in eel research.

Finally, an effective Eel Management Plan supports both eel fishery and eel conservation, and Mediterranean coastal lagoons are suitable environments. With regards to Italy, the proposed European Restocking Areas lie in North Adriatic Sea:

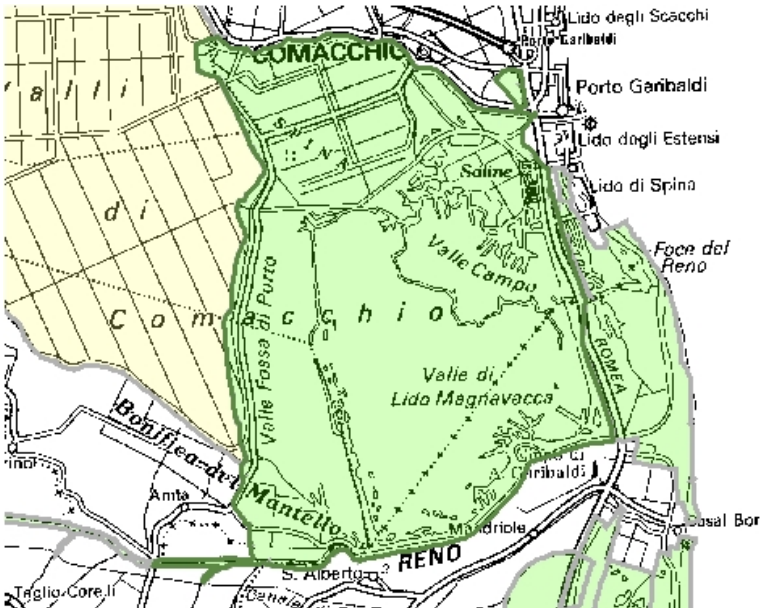


Figure 6.9.3. The Natura 2000 site IT4060002: Comacchio lagoon.

The Comacchio lagoon is a well-know historical eel breeding area (Tesch, 2003) and nowadays a public-private partnership can realize the dream to restore the eel population at the historical level of a Century ago.

6.10 Acknowledgement

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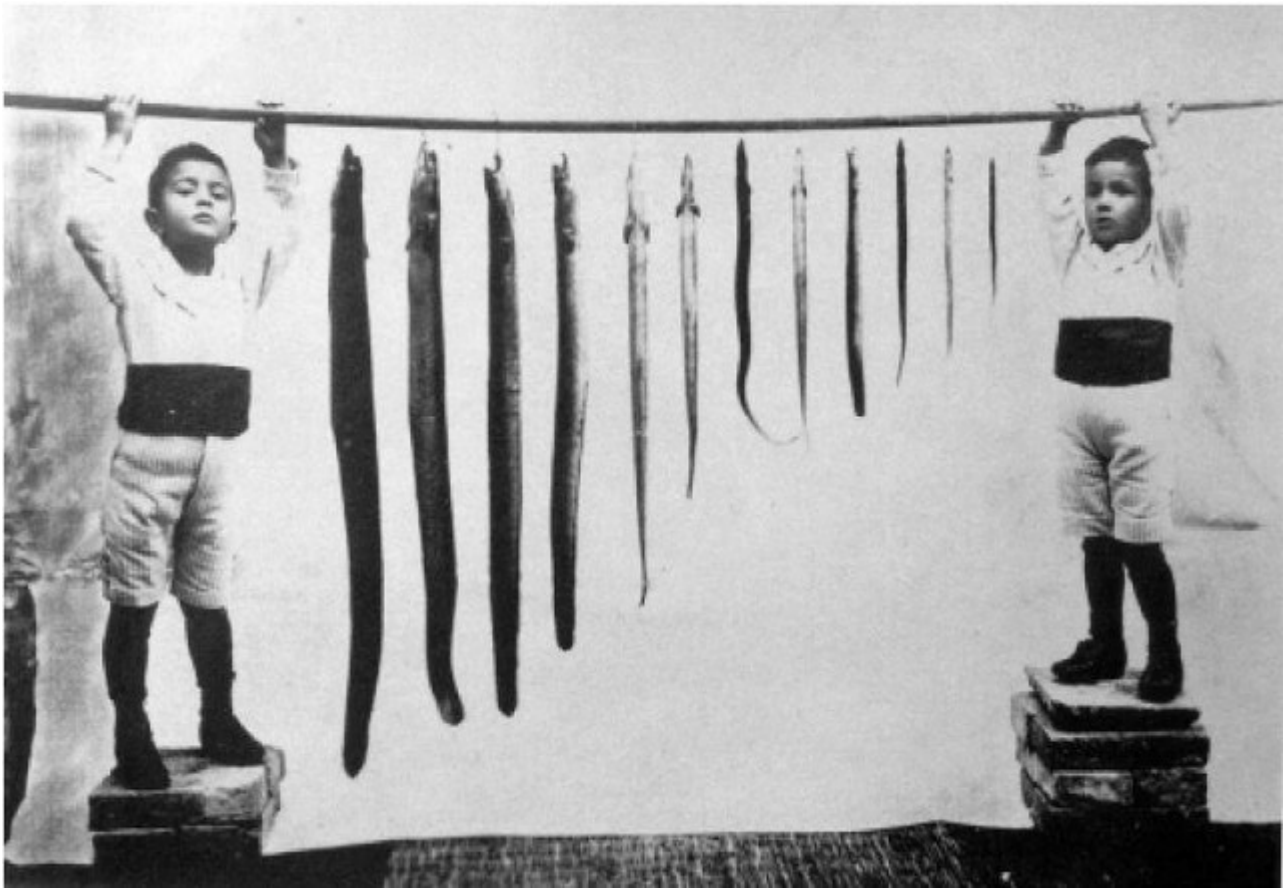
Annex I - Estimates on eels



List of tables

Terms and Units

L	total body length	[mm]
W	total body weight	[g]
FL	pectoral fin length	[mm]
MD	mean eye diameter	[mm]

ASR	Adequate Sampling Ratio
CF	Condition Factor (Fulton, 1904)
EI	Eye Index (Pankhurst, 1983)
SI	Silver Index (Durif, 2009)

OTO	age reading by otolith	[years]
SCA	age reading by fish scale	[years]

Group A – 2009

	L	W	FL	MD	SI	ASR	IE	CF
1	835	1064	40.25	9.77	4	1.00	8.29	0.18
2	770	789	36.86	9.34	3	1.24	8.29	0.17
3	830	1356	39.32	11.00	4	0.78	10.73	0.24
4	890	1595	43.35	10.89	4	0.71	10.09	0.23
5	870	1330	39.34	10.15	4	0.83	8.75	0.20
6	780	886	35.53	10.68	4	1.12	10.26	0.19
7	840	1455	38.78	9.56	4	0.74	7.77	0.25
8	925	1650	42.84	9.57	4	0.71	7.06	0.21
9	900	1750	41.53	8.84	4	0.66	6.45	0.24
10	800	1215	37.49	7.88	4	0.84	5.66	0.24
11	705	630	29.90	6.46	2	1.43	4.25	0.18
12	860	1325	35.04	9.73	4	0.83	8.10	0.21
13	830	1315	37.07	9.99	4	0.80	9.10	0.23
14	725	740	39.52	8.99	5	1.25	8.29	0.19
15	835	1095	37.45	8.51	3	0.97	6.41	0.19
16	840	1305	41.46	9.90	4	0.82	8.40	0.22
17	800	1020	37.98	9.78	4	1.00	8.85	0.20
18	795	1130	38.12	8.73	4	0.90	7.03	0.22
19	990	2035	38.21	11.44	4	0.62	9.89	0.21
20	825	1200	36.34	9.89	4	0.88	8.86	0.21
21	895	1425	47.34	8.96	4	0.80	6.71	0.20
22	830	1405	40.50	9.36	4	0.75	7.56	0.25
23	780	1160	37.77	11.07	4	0.86	11.41	0.24
24	790	1220	34.25	9.24	4	0.82	7.88	0.25
25	780	1115	37.35	9.62	4	0.89	8.15	0.23
26	865	1230	41.34	9.25	4	0.90	7.23	0.19
27	825	1210	41.78	10.86	4	0.87	10.72	0.22
28	815	1375	39.79	9.89	4	0.76	8.67	0.25
29	820	1135	34.28	9.89	4	0.92	9.37	0.21
30	830	1210	36.23	10.08	4	0.87	8.92	0.21
31	925	1710	43.20	10.10	4	0.69	8.23	0.22
32	890	1390	35.92	10.53	4	0.82	9.09	0.20
33	815	1165	38.89	8.86	4	0.89	7.17	0.22
34	770	1025	32.38	9.02	4	0.96	7.80	0.22
35	880	1565	39.61	11.47	4	0.72	10.99	0.23
36	820	1410	36.89	10.65	4	0.74	10.30	0.26
37	815	1225	39.57	9.59	4	0.85	8.27	0.23
38	820	1210	36.79	10.11	4	0.86	9.78	0.22
39	810	1250	40.28	10.35	4	0.83	10.38	0.24
40	810	1190	36.15	9.48	4	0.87	8.70	0.22
41	840	1275	35.34	9.01	4	0.84	7.58	0.22
42	835	1195	35.81	10.33	4	0.89	10.03	0.21
43	860	1605	40.79	10.14	4	0.68	9.39	0.25
44	775	860	37.01	10.25	5	1.15	10.64	0.18
45	815	1190	33.64	9.45	4	0.87	8.59	0.22
46	780	1005	39.76	9.72	4	0.99	9.51	0.21
47	865	1540	39.78	10.47	4	0.72	9.95	0.24
48	830	1290	39.37	9.96	4	0.82	9.38	0.23
49	910	1715	40.64	10.69	4	0.68	9.85	0.23
50	850	1365	36.78	10.02	4	0.79	9.27	0.22
51	815	1355	38.33	9.76	4	0.77	9.17	0.25
52	790	1170	41.18	10.31	4	0.86	10.56	0.24
53	850	1580	39.00	10.30	4	0.69	9.80	0.26
54	770	1115	36.80	10.30	4	0.88	10.82	0.24
55	810	1105	41.70	10.10	4	0.93	9.89	0.21
56	765	930	35.00	10.30	4	1.05	10.89	0.21
57	810	1085	32.10	9.10	4	0.95	8.03	0.20
58	855	1225	39.90	9.50	4	0.89	8.29	0.20
59	750	890	31.90	9.00	3	1.07	8.48	0.21
60	910	1530	42.10	10.05	4	0.76	8.71	0.20
61	895	1840	37.00	10.05	4	0.62	8.86	0.26
62	820	1455	37.00	9.75	4	0.72	9.10	0.26
63	850	1295	41.00	8.10	4	0.84	6.06	0.21
64	880	1445	41.20	9.35	4	0.78	7.80	0.21
65	790	925	36.90	9.15	3	1.09	8.32	0.19
66	895	1540	39.40	9.95	4	0.74	8.68	0.21
67	830	1285	35.40	9.65	4	0.82	8.81	0.22
68	760	1010	37.90	7.75	3	0.96	6.20	0.23
69	870	1665	39.00	9.15	4	0.67	7.55	0.25
70	725	795	32.50	9.40	4	1.16	9.57	0.21
71	400	90	15.00	3.95	1	5.66	3.06	0.14
72	845	1245	39.00	8.70	4	0.86	7.03	0.21
73	785	915	31.40	7.15	3	1.09	5.11	0.19
74	780	1095	33.10	8.30	4	0.91	6.93	0.23
75	815	1075	37.80	8.25	3	0.97	6.56	0.20
76	840	1300	39.60	9.40	4	0.82	8.26	0.22
77	835	1315	43.00	9.05	4	0.81	7.70	0.23
78	780	1025	34.50	7.95	3	0.97	6.36	0.22
79	465	135	14.60	4.40	1	4.39	3.27	0.13
80	885	1395	38.60	9.10	4	0.81	7.35	0.20
81	785	890	31.80	8.00	3	1.12	6.40	0.18
82	625	615	35.10	9.20	5	1.29	10.63	0.25
83	785	910	35.10	7.55	3	1.10	5.70	0.19
84	795	1250	35.90	7.85	4	0.81	6.08	0.25
85	730	900	31.90	8.15	3	1.03	7.14	0.23
86	815	1065	36.20	8.30	3	0.97	6.64	0.20
87	880	1645	39.40	9.30	4	0.68	7.72	0.24
88	770	1190	36.80	7.95	4	0.82	6.44	0.26
89	900	1590	39.70	10.10	4	0.72	8.90	0.22
90	890	1540	40.30	8.75	4	0.74	6.75	0.22
91	815	1040	38.10	7.95	3	1.00	6.09	0.19
92	750	805	30.70	7.90	3	1.19	6.53	0.19
93	795	1160	36.90	8.45	4	0.87	7.05	0.23
94	765	945	35.30	8.15	3	1.03	6.82	0.21
95	720	775	39.00	7.80	3	1.18	6.63	0.21
96	520	260	22.50	4.80	2	2.55	3.48	0.18

Group A – 2010

	L	W	FL	MD	SI	ASR	IE	CF
1	865	1265	38.8	12.15	4	0.87	12.58	0.20
2	800	2490	46.4	12.20	4	0.41	12.20	0.49
3	895	1390	44.6	11.20	4	0.82	10.33	0.19
4	915	1545	40.9	10.55	4	0.75	8.97	0.20
5	850	1580	39.7	9.60	4	0.69	7.99	0.26
6	900	1910	41.4	10.80	4	0.60	9.71	0.26
7	860	1255	34.2	10.80	4	0.87	10.16	0.20
8	920	1770	44.0	10.25	4	0.66	8.75	0.23
9	800	1140	36.2	10.35	4	0.89	9.96	0.22
10	820	1220	36.8	9.80	4	0.86	8.82	0.22
11	875	1540	40.3	11.50	4	0.72	11.56	0.23
12	850	1475	43.5	9.55	4	0.73	7.77	0.24
13	765	1030	34.0	9.60	4	0.95	9.07	0.23
14	860	1535	36.5	12.05	4	0.71	13.20	0.24
15	825	1285	38.5	11.90	4	0.82	12.80	0.23
16	850	1165	37.2	10.45	4	0.93	9.10	0.19
17	870	1495	39.1	11.00	4	0.74	10.52	0.23
18	800	1150	40.7	11.45	4	0.89	12.70	0.22
19	860	1445	37.9	10.35	4	0.76	9.45	0.23
20	805	1210	37.5	10.25	4	0.85	10.00	0.23
21	870	1450	35.8	10.90	4	0.76	11.18	0.22
22	905	1655	38.0	10.65	4	0.62	10.46	0.32
23	925	1770	39.8	10.00	4	0.64	8.77	0.25
24	865	1410	40.4	10.60	4	0.83	8.75	0.18
25	935	1665	40.7	10.55	4	0.65	9.85	0.27
26	875	1650	40.6	10.70	4	0.69	9.99	0.23
27	865	1390	36.3	11.50	4	0.79	11.55	0.22
28	845	1380	41.0	11.00	4	0.85	9.86	0.18
29	750	900	33.4	9.75	3	1.13	8.62	0.18
30	865	1560	42.5	11.55	4	0.67	12.28	0.28
31	850	1505	38.4	10.75	4	0.74	10.03	0.22
32	780	1130	34.8	10.40	4	0.96	9.80	0.18
33	815	1260	40.0	10.85	4	0.77	12.14	0.28
34	810	1100	39.0	9.60	3	1.00	8.24	0.17
35	940	1765	40.5	11.25	4	0.60	11.46	0.31
36	920	1610	38.6	10.70	4	0.67	10.38	0.26
37	880	1545	39.6	12.10	4	0.72	12.67	0.23
38	890	1755	38.6	11.30	4	0.58	12.53	0.34
39	910	1620	38.3	10.70	4	0.68	10.45	0.25
40	875	1510	41.4	11.70	4	0.68	13.35	0.29
41	890	1885	40.4	11.65	4	0.60	11.97	0.27
42	870	1680	42.0	11.30	4	0.66	11.52	0.26
43	850	1465	36.0	10.85	4	0.74	10.87	0.24
44	775	1020	30.8	9.60	4	0.97	9.33	0.22
45	690	680	32.5	9.25	5	1.29	9.73	0.21
46	830	1185	33.6	10.30	4	0.89	10.03	0.21
47	955	2225	45.0	11.10	4	0.55	10.13	0.26
48	830	1480	40.5	10.20	4	0.71	9.84	0.26
49	790	1085	30.7	9.75	4	0.93	9.45	0.22
50	900	1595	38.1	10.85	4	0.72	10.27	0.22
51	790	1145	40.2	10.55	4	0.88	11.06	0.23
52	815	1265	38.5	10.85	4	0.82	11.34	0.23
53	815	1195	35.7	10.05	4	0.87	9.73	0.22
54	810	1380	33.3	10.50	4	0.75	10.68	0.26
55	850	1495	38.8	11.65	4	0.72	12.53	0.24
56	915	1670	38.9	11.40	4	0.70	11.15	0.22
57	875	1525	41.2	11.10	4	0.73	11.05	0.23
58	820	1335	36.8	10.10	4	0.78	9.77	0.24
59	885	1540	34.5	10.75	4	0.73	10.25	0.22
60	870	1525	38.1	11.25	4	0.73	11.42	0.23
61	785	1300	38.2	10.40	4	0.77	10.82	0.27
62	840	1430	36.1	10.85	4	0.75	11.00	0.24
63	865	1395	40.7	10.65	4	0.79	10.29	0.22
64	820	1370	36.9	9.50	4	0.76	8.64	0.25
65	815	1110	35.6	10.15	4	0.94	9.92	0.21
66	840	1360	37.8	9.00	4	0.79	7.57	0.23
67	920	1720	42.3	11.35	4	0.68	10.99	0.22
68	770	950	35.2	9.55	4	1.03	9.30	0.21
69	835	1215	36.8	10.05	4	0.88	9.50	0.21
70	800	1130	35.3	10.45	4	0.90	10.72	0.22
71	935	1500	37.9	10.65	4	0.79	9.52	0.18
72	805	1230	37.3	10.30	4	0.83	10.35	0.24
73	775	1100	37.3	9.85	4	0.90	9.83	0.24
74	870	1510	38.5	10.05	4	0.73	9.11	0.23
75	780	1100	35.4	9.65	4	0.90	9.37	0.23
76	765	1185	33.6	9.75	4	0.82	9.75	0.26
77	725	805	35.8	10.10	5	1.15	11.05	0.21
78	850	1220	36.7	11.50	4	0.89	12.21	0.20
79	860	1445	40.9	10.70	4	0.76	10.45	0.23
80	840	1195	37.0	10.60	4	0.90	10.50	0.20
81	855	1495	38.4	10.75	4	0.73	10.61	0.24
82	895	1565	37.1	9.70	4	0.73	8.25	0.22
83	850	1330	43.8	10.20	4	0.81	9.61	0.22
84	820	1210	38.1	11.10	4	0.86	11.80	0.22
85	850	1295	40.8	10.50	4	0.84	10.18	0.21
86	865	1555	43.5	11.25	4	0.71	11.49	0.24
87	800	1215	38.8	9.70	4	0.84	9.23	0.24
88	935	1670	42.7	10.45	4	0.71	9.17	0.20
89	845	1320	41.3	10.80	4	0.82	10.84	0.22
90	790	1180	36.5	10.70	4	0.85	11.38	0.24
91	850	1650	38.2	10.85	4	0.66	10.87	0.27
92	875	1460	47.5	10.55	4	0.76	9.99	0.22
93	845	1580	43.8	11.45	4	0.68	12.18	0.26
94	865	1500	35.5	10.00	4	0.73	9.08	0.23
95	800	1190	37.3	10.10	4	0.86	10.01	0.23
96	750	1225	40.4	11.30	4	0.78	13.36	0.29
97	880	1615	44.5	10.55	4	0.69	9.93	0.24
98	830	1215	41.4	10.90	4	0.87	11.24	0.21
99	860	1605	37.4	9.30	4	0.68	7.89	0.25
100	855	1345	40.2	10.60	4	0.81	10.32	0.22

Group A – 2011

	L	W	FL	MD	SI	ASI	EI	CF
1	835	1325	40.6	10.05	4	0.80	9.08	0.23
2	830	1135	36.4	10.10	4	0.93	9.65	0.20
3	840	1410	36.4	10.10	4	0.76	8.88	0.24
4	775	1120	35.4	8.30	4	0.88	6.48	0.24
5	770	1000	30.2	9.35	4	0.98	8.30	0.22
6	670	720	32.3	9.10	4	1.19	9.60	0.24
7	760	955	33.9	9.50	4	1.01	9.32	0.22
8	850	1330	35.9	10.70	4	0.81	10.28	0.22
9	800	1100	34.1	9.55	4	0.93	8.35	0.21
10	775	930	33.7	9.60	4	1.06	8.95	0.20
11	740	950	29.6	8.30	3	0.99	7.05	0.23
12	840	1320	40.3	10.85	4	0.81	10.45	0.22
13	760	1020	37.5	10.50	4	0.95	11.28	0.23
14	700	790	33.2	9.40	4	1.13	9.49	0.23
15	750	920	33.5	9.70	4	1.04	9.85	0.22
16	740	670	30.5	8.40	3	1.41	7.05	0.17
17	730	865	36.6	8.70	3	1.08	8.33	0.22
18	795	1085	36.3	10.40	4	0.93	10.58	0.22
19	740	900	34.9	9.95	4	1.05	9.83	0.22
20	830	1145	39.7	9.75	4	0.92	8.94	0.20
21	730	740	32.7	8.55	3	1.44	6.52	0.19
22	740	900	38.2	9.30	3	1.17	8.18	0.22
23	840	1360	39.8	10.80	4	0.79	10.80	0.23
24	640	555	29.1	8.15	3	1.78	6.60	0.21
25	860	1250	37.6	9.75	4	0.78	9.64	0.20
26	770	980	30.5	9.05	4	0.87	9.33	0.21
27	840	1255	34.6	8.80	4	0.77	7.55	0.21
28	690	670	29.4	7.50	3	1.62	5.13	0.20
29	870	1520	41.5	10.85	4	0.67	10.97	0.23
30	670	600	31.5	9.55	3	1.65	8.90	0.20
31	825	1505	40.0	10.60	4	0.63	11.81	0.27
32	780	995	35.3	9.90	3	1.08	8.88	0.21
33	640	585	26.6	8.40	3	1.65	7.12	0.22
34	840	1355	42.5	8.65	4	0.66	8.15	0.23
35	760	920	32.6	9.10	4	1.04	8.29	0.21
36	810	1130	36.7	10.25	4	0.83	10.66	0.21
37	780	1190	36.0	10.45	4	0.78	11.13	0.25
38	700	910	32.2	8.60	3	1.11	7.30	0.27
39	765	990	34.8	9.60	4	0.95	9.78	0.22
40	735	985	32.5	8.85	3	1.07	7.41	0.25
41	710	795	34.9	10.35	5	1.14	11.84	0.22
42	805	960	34.9	9.95	4	1.07	9.65	0.18
43	770	1005	33.5	8.45	3	0.98	7.28	0.22
44	780	1110	37.0	10.15	4	0.90	10.37	0.23
45	770	1020	37.8	8.90	4	0.96	8.08	0.22
46	780	905	34.9	8.65	3	1.10	7.53	0.19
47	720	690	31.8	9.45	3	1.33	9.74	0.18
48	810	1385	40.3	10.95	4	0.75	11.62	0.26
49	820	1070	40.5	9.50	4	0.98	8.64	0.19
50	740	915	38.8	9.45	4	1.03	9.47	0.23
51	730	860	35.2	10.25	4	1.08	11.30	0.22
52	840	1330	35.5	11.40	4	0.80	12.15	0.22
53	765	1105	36.0	7.95	4	0.88	6.49	0.25
54	800	1135	33.3	8.20	3	0.90	6.60	0.22
55	795	875	34.9	9.30	3	1.16	8.54	0.17
56	710	725	39.0	9.45	5	1.25	9.87	0.20
57	830	1425	38.3	10.40	4	0.74	10.23	0.25
58	820	1230	36.2	10.50	4	0.85	10.55	0.22
59	770	895	32.3	10.10	4	1.10	10.40	0.20
60	715	835	35.0	9.05	4	1.09	8.99	0.23
61	760	935	34.6	9.15	4	1.04	8.65	0.21
62	780	1100	36.5	9.60	4	0.90	9.28	0.23
63	690	710	32.2	8.70	3	1.24	8.61	0.22
64	780	1180	36.3	9.45	4	0.84	8.99	0.25
65	730	750	32.1	10.15	5	1.24	11.08	0.19
66	730	935	30.4	7.80	3	0.99	6.54	0.24
67	820	1090	34.5	10.10	4	0.96	9.77	0.20
68	750	840	31.2	9.40	3	1.14	9.25	0.20
69	770	980	34.2	9.90	4	1.00	9.99	0.21
70	780	885	29.8	8.60	3	1.12	7.44	0.19
71	835	1255	35.6	10.40	4	0.85	10.17	0.22
72	825	1075	34.5	9.45	4	0.98	8.50	0.19
73	765	1055	33.9	10.10	4	0.92	10.47	0.24
74	750	1000	35.6	9.55	4	0.96	9.55	0.24
75	840	1320	32.2	9.45	4	0.81	8.35	0.22
76	780	1090	38.3	8.70	4	0.91	7.62	0.23
77	860	1185	35.1	10.10	4	0.92	9.31	0.19
78	790	1215	39.0	10.40	4	0.83	10.75	0.25
79	835	1240	40.4	10.35	4	0.86	10.07	0.21
80	715	905	37.4	9.65	4	1.01	10.22	0.25
81	810	1265	40.3	9.85	4	0.82	9.40	0.24
82	805	1140	32.3	10.15	4	0.90	10.05	0.22
83	830	1455	41.4	9.85	4	0.73	9.18	0.25
84	650	550	27.0	8.00	3	1.51	7.73	0.20
85	805	1035	35.5	9.35	4	0.99	8.53	0.20
86	670	715	33.5	7.95	3	1.19	7.41	0.24
87	720	810	34.5	9.00	3	1.13	8.83	0.22
88	810	1160	37.7	9.05	4	0.89	7.94	0.22
89	685	775	30.9	8.65	3	1.13	8.57	0.24
90	780	1025	36.0	10.40	4	0.97	10.89	0.22
91	790	1140	38.7	9.85	4	0.88	9.64	0.23
92	850	1125	40.0	10.75	4	0.96	10.67	0.18
93	760	815	34.2	8.25	3	1.19	7.03	0.19
94	720	890	35.5	9.50	4	1.03	9.84	0.24
95	915	1755	42.5	11.00	4	0.66	10.38	0.23
96	630	650	30.4	8.75	4	1.23	9.54	0.26
97	750	800	33.2	8.20	3	1.19	7.04	0.19
98	800	1050	36.5	9.60	4	0.97	9.04	0.21
99	790	1165	39.3	10.15	4	0.86	10.24	0.24
100	775	895	36.7	9.05	3	1.10	8.30	0.19

Group B – 2009

	L	W	FL	MD	SI	ASR	EI	CF	LSI	GSI	RBC	WBC	HT	OTO	SCA
1	795	1104	37.22	9.29	4	0.92	8.52	0.22	1.21	1.91	1195000	66000	38	6	6
2	890	1428	33.05	9.26	4	0.79	7.55	0.20	1.25	1.73	1300000	60750	44	7	7
3	830	1406	39.00	9.89	4	0.75	9.24	0.25	1.18	1.53	1120000	105750	36	6	6
4	870	1357	39.40	10.50	4	0.82	9.95	0.21	1.06	1.08	2215000	76500	36	10	10
5	845	1186	34.34	10.49	4	0.91	10.21	0.20	1.10	1.80	2855000	54500	58	7	7
6	850	1242	39.75	10.57	4	0.87	10.31	0.20	1.24	1.48	1655000	127250	42	7	7
7	880	1181	39.60	9.74	4	0.95	8.45	0.17	1.28	1.80	2535000	49250	37	NA	NA
8	880	1571	45.77	9.44	4	0.71	7.94	0.23	1.21	1.13	NA	NA	NA	NA	NA

Group B – 2010

	L	W	FL	MD	SI	ASR	EI	CF	LSI	GSI	RBC	WBC	HT	OTO	SCA
1	930	1250	36.9	10.70	4	0.95	9.66	0.16	1.02	1.70	1575000	37000	37.5	6	7
2	770	1250	38.1	9.95	4	0.78	10.09	0.27	0.97	1.25	1920000	37000	31	6	5
3	835	1650	43.2	10.30	4	0.64	9.97	0.28	1.03	1.41	1635000	25500	41	8	7
4	800	1400	38.9	9.45	4	0.73	8.76	0.27	0.89	1.45	2700000	42500	41	8	7
5	790	1050	38.6	10.65	4	0.96	11.27	0.21	0.97	1.37	2660000	65500	35	6	6
6	805	1200	34.5	10.05	4	0.85	9.85	0.23	0.84	1.51	1540000	49000	38	7	7
7	905	1700	39.2	10.25	4	0.68	9.11	0.23	1.15	1.67	1760000	48500	34	7	7
8	910	1400	39.9	10.90	4	0.83	10.25	0.19	1.00	1.71	1390000	53500	28	8	7

Group B – 2011

	L	W	FL	MD	SI	ASR	EI	CF	LSI	GSI	RBC	WBC	HT	OTO	SCA
1	820	1860	42.2	8.85	4	0.56	7.50	0.34	NA	NA	1915000	44500	28	7	8
2	840	1400	39.6	10.37	4	0.76	10.05	0.24	NA	2.13	1555000	54500	24	6	6
3	890	1560	43.6	11.70	4	0.73	12.07	0.22	NA	1.62	1740000	81000	35	6	7
4	935	1690	41.4	11.40	4	0.70	10.91	0.21	NA	1.45	3355000	23500	28	6	7
5	830	1377	34.9	9.45	4	0.77	8.45	0.24	NA	1.49	1290000	57500	27	8	7
6	910	1817	47.7	11.10	4	0.64	10.63	0.24	NA	1.96	1650000	74000	33	6	7
7	790	1168	34.4	9.60	4	0.86	9.16	0.24	NA	2.07	1500000	79500	27	6	6
8	840	1408	41.6	10.85	4	0.76	11.00	0.24	NA	2.00	1830000	88000	33	6	6

Annex II - Sustainable eel fishery in the Comacchio lagoon

Boy with female eels in Comacchio.

Introduction

The conservation of European eels is widely discussed in scientific literature as well as in aquaculture. Most of topics are related to a single target (i.e. eel conservation or farming), while a improvement of capability should be considered for an effective sustainable eel fishery. This PhD thesis shows that Mediterranean coastal lagoons are suitable habitats for eel restocking, both for management facilities and stock's features. Furthermore, since 2004, in the Comacchio lagoon the protected area management body (Po Delta Park, nowadays Authority for Biodiversity Conservation - Delta of Po river), is working for habitat conservation and sustainable eel fishery, also trying to develop a method for the self-financing of eel fishery. The Authority is in charge both for harvest of fish and food processing (Marinated eel, see Annex III).

Fostering of local products

In order to foster high quality food production, is important to evaluate the commercial potential provided by food processing and by promotion of production's area.

A "value-added" is a food products that is converted from raw product through processes that give the resulting product an "incremental value" in the market place (Steven, 2008).

The financial advantages provided are due to a availability of customers to pay more a products that a) use high quality raw materials, b) is produced in a protected area or in a particular area (coast, mountain, landscape,…).

Actually, the bottle-neck of sustainable eel fishery is the financial capability (both for public bodies and private companies) for restocking.

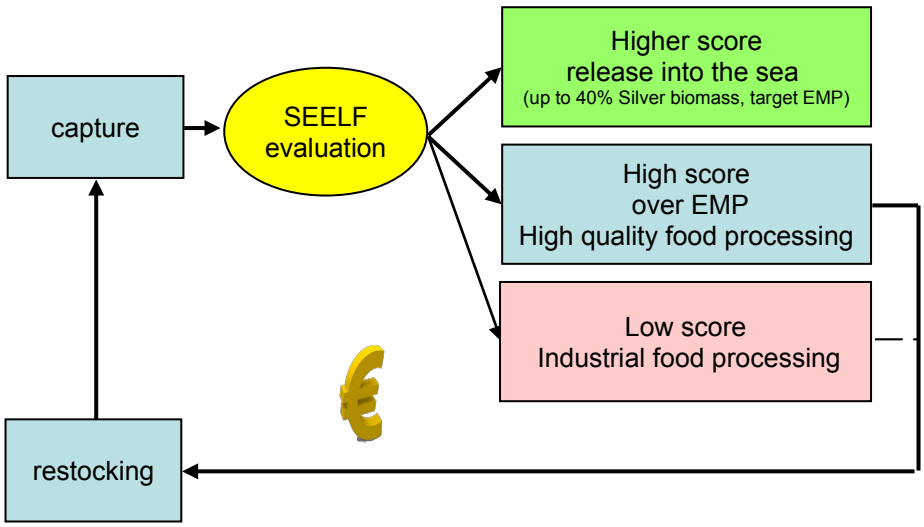


Figure II.1. Comacchio model for the self-financing of sustainable eel fishery.

The figure II.1 shows the organisational model for self-financing of sustainable eel fishery: the fish captured at the *lavoriero*, is evaluated with the SEELF Index (version A) and, because of score, release to the open sea (for natural restocking, according to EMP) or used in local fishery economy. Samples with higher score is used for food processing into typical product (high quality) and others are used in different low quality chain product.

With a suitable marketing, the typical product's cost can include a quote for funding environmental management and eels restocking, while industrial product can provided new jobs.

This approach aims to support both public bodies (protected area management authorities) and private firms (food processing, tourism services), in order to boost the sustainable local development of coastal area.

Regarding local development, high quality food products are important because the producers are not only interchangeable (and exploitable) input suppliers, but they are "strategic partners" with rights and responsibilities related to value chain information, risk-taking, governance, and decision-making (Steven, 2008).

The need of novel approach for fishery management was recently discussed by Battaglia et al (2010); for the Aeolian islands they suggest to managers to apply an integrated approach to coastal zone management, by (i) appropriate evaluation of resources at market level (e.g. underutilized species), (ii) support to "fishing tourism" initiatives, (iii) conflict resolution between fishery and biodiversity conservation and (iv) conflict resolution between Marine Protected Area management and tourism demands and fishermen's livelihoods.

A sustainable eel fishery can foster all aquaculture's products as I going to be in the Comacchio area. In fact, although very limited in number, the eel product is an exceptional brand that improve the commercial capability of all fish industries.

Annex III - Production of Marinated eel in the Comacchio area

Marinated Eel of Comacchio the typical product of Po Delta Park (ph. Po Delta Park archive).

A. Production of Traditional Marinated Eel in the Comacchio area

The Comacchio lagoon hosts an extensive farm of European eel (Tesch, 2003) and is included in a NATURA2000 site, in a regional protected area called Po Delta and in the UNESCO World's Heritage site of “Ferrara, city of Renaissance and its Po Delta”. During the last centuries, the food industry based on this fish has developed the “marinated eel”, that has became a typical product and is widely famous in the world.

Figure III.1. The label of the traditional marinated eel of Comacchio

The Traditional Marinated Eel of the Comacchio Lagoon is produced with “silver eel” (mature female specimen) cooked at fireplace and stored in can, with a brine of vinegar, water and salt; the canned fish have to be stored at 4°C and the whole process was standardized in 2004. The quality of the product, behind factory's process, is strongly influenced by two main factor: (1) quality of harvested fish and (2) storage at constant low temperature of cans. The quality of captured fish is evaluated by index (SEELF), as described in thesis.

The marinated eel is produced by the Po Delta Park authority and is part of a larger programme for conservation of European eel, that includes a self-financing of protected area by commerce of typical products (see Annex II). The product is recognized by the Italian law (national list of traditional agriculture products, decree June 16th 2008), SlowFood (Presidium of Traditional Pickled Eel of Lagoon of Comacchio) and Italian Nature Park Federation (Atlas of typical products of Parks, in association con SlowFood).

Figure III.2. Logos for high quality food products of the Po Delta Park (left) and SlowFood Presidium (right).

B. Food processing

The production of Marinated eel, following the ancient recapture, involve exclusively feral eels, fast decapitation and cooking at fireplace, as shown in figure AIII.3.

Figure III.3. Food processing of marinated eel in Comacchio (ph. Po Delta Park archive)

The food processing is based on human knowledge of environment of lagoon, harvest of eels and plant management. The applicable legislation follow the framework of European food safety, i.e. Regulation (EC) No 178/2002 containing general principles of food law, which explains food safety procedures and establishes the European Food Safety Authority, Regulation (EC) No 852/2004 on the hygiene of foodstuffs, Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin in order to guarantee a high level of food safety and public health, Regulation (EC) No 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption and Regulation (EC) No 882/2004 reorganising official controls on foodstuffs and feeding stuffs so as to integrate controls at all stages of production and in all sectors.

C. HACCP system

The Hazard Analysis and Critical Control Point (HACCP) is a method that identifies, evaluates and controls hazards that are significant for food safety (Codex Alimentarus, 2003). The HACCP systems have to define adequate control parameters; for marinated eel, they are:

a. fish's temperature at the end of cooking process (>85°C)
b. brine's pH (pH<4,5; FAO, 1985)
c. storage temperature of product/primary packaging (4°C)

In order to provide an appropriated food safety, these requirements were scientifically proved by a study performed by Stazione Sperimentale delle Conserve Alimentari (SSICA, Ichthyological Laboratory, Parma - Italy), ARPA Emilia-Romagna (Ichthyological Research Unit, Ferrara - Italy) and University of Bologna. As final result, the "best before" statement was evaluated in 12 months.

The HACCP system is the basis for an excellent production with respect of food safety requirement; following table summarizes the main targets:

Food processing rules	Imposed by Marinated Eel Disciplinary of Production, 2004	Suggested by UNIBO-CIRSA proposal, 2012
Admitted capture tools	Lavoriero and fyke-nets	Lavoriero only
Stunning method	24h in air	Electro-stunning or anaesthesia with adequate substance (to define)
Fish evaluation	Non-parametric evaluation of silvering	SEELF Index (A version)
Admitted fish sizes	300-350g/each	Selected by ASR

Table III.1. Food processing requirements of Marinated Eel: in use (left) and proposed (right).

D. Organic farming

Codex Alimentarius define “organic” a products that have been produced in accordance with organic production standards and properly certified by a certification body or authority. Organic agriculture is based on best practises, that reduce chemicals as well as external inputs. Although organic agriculture cannot ensure a completely free of residues product (also due to general environmental pollution) it aims to optimize the human health and a sustainable use of natural resources (Codex Alimentarius, 1999).

Organic farming is part of a large supply chain, including food processing, distribution and retailing sectors and, ultimately, customers. Each link of the supply chain have to be designed to deliver benefits for citizens, across a wide range of topics, such as environmental protection, animal welfare, food safety, consumer confidence and economy.

The EC Regulation No.834/2007 provides the basis for the sustainable development of organic production while ensuring the effective functioning of organic products in the European market. The EC Regulation No.710/2009 (Organic production of eels in coastal lagoons – Annex XIII, section 4) gives some details on habitats (ponds) and maximum density (4kg/m^3) for organic productions.

Figure III.4. Logo of organic product in EU

The Disciplinary of Production, if modify as suggested in this thesis, fits the major requirements for the recognition of organic production:

☑	origin of animals
☑	breeding of animals
☑	animal wellness*
☑	training of employee
☑	environmental condition
☑	low environmental impact fishery
☑	rules on productors

Table III.2. Requirements for Organic Product, as imposed by the EU Regulation No 834/2007, EU Regulation No 710/2009 (in act since 2010) [Organic production of eels in coastal lagoons – Annex XIII, section 4]. * Also foresee by EU Regulation No 1099/2009 on animal wellness.

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Evaluation of silver European eel (*Anguilla anguilla*) for the implementation of an effective Eel Management Plan in Mediterranean coastal lagoons.

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