Estuarine, Coastal and Shelf Science 299 (2024) 108660

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Silvering process of female European eel in the north Adriatic: Who is really ready to migrate?

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A R T I C L E I N F O

Keywords:
Anguilla Anguilla
Gonadal maturation
Artificial reproduction
Restoration
Estuarine
Lagoons

A B S T R A C T

During the 1970s and 2010, the population of *Anguilla anguilla* declined significantly, and the species is now classified as a critically endangered species by the International Union for Conservation of Nature (IUCN). Considering its social, economic, and ecological importance, it is necessary to understand the biological mechanism that controls population dynamics, in particular the passage from the resident phase to the migratory phase. During this phase of their life cycle, the environment plays a key role in the maturation of the spawners which will eventually undertake a long spawning migration to the Sargasso Sea.

An assessment of the morphological characteristics of eels during their silvering process and reproductive activity was conducted by collecting 1513 female eels from seven lagoons in the North Adriatic Sea.

Morphometric analyses show that eels classified as pre-migratory according to the Silver Index are histologically (65% of eels with oocytes in the most advanced stage of development) and morphologically ready to migrate. Accordingly, pre-migrant eels treated with hormone induction performed similarly to migrant eels. They mated and spawned spontaneously in the tank with a percentage of 81.0%, and 88.2% of them produced viable eggs. The results showed how the North Adriatic lagoons could present favourable conditions that permit the fast development of females with high reproductive potential. This data can be used to develop successful strategies to conserve and manage eel populations.

1. Introduction

The European eel, *Anguilla anguilla* (Linnaeus, 1758) is a catadromous teleost, which exhibits a complex life cycle with spawning in the ocean and growing up in continental waters (Tesch, 1977; Bonhommeau et al., 2010). This species has been subject to extensive scientific inquiry due to its enigmatic natural ecology. The available information indicates that the stock has declined severely in most of its distribution areas and the population has decreased dramatically between the late 1970s and 2010 (Drouineau et al., 2018; ICES, 2018). Since then, it is now at its historical minimum and classified as "Critically Endangered" according to the international union for conservation of nature (Pike et al., 2020; Dekker, 2003; ICES, 2021). To address the bad state of the stock, in 2007, the European Union adopted a management plan for the recovery of the European eel stock through an EU regulation (EC, 2007). Since 2018, 3-month fishing closures have been introduced as well at the EU level (EU, 2018). Recently, ICES recommended zero catches in all habitats in 2023. As such, it applies to both recreational and commercial catches as well as glass eel restocking and aquaculture (ICES, 2022).

Several factors have been cited to explain the rapid decline of the European eel stock, including anthropogenic factors such as overfishing, pollution, habitat degradation, parasites, and diseases, as well as environmental factors such as climate change and ocean changes (Palstra et al., 2006, 2007; Robinet and Feunteun, 2002; Durif et al., 2003; Dekker, 2019).

Considering its social, economic, and ecological importance, measures have been taken to monitor and restock the remaining populations. For this reason, it is necessary to evaluate the remaining stock, their environmental conditions, and to better understand the biological mechanisms that control the population dynamics of the species, e.g., the morphological and physiological transformation of the catadromous species in preparation of the migration to their mating and spawning area. There is an ongoing scientific discussion if the eels, stationed in continental waters for a range of 10–18 years (with a lot of variation across Europe) undergo a metamorphosis event, or a pubertal event (Aroua et al., 2006) from resident to migrant eel. The morphological and physiological changes during these events are linked to the transition from freshwater to saltwater lifestyle, and to the preparation for the long migration, which is even longer in the case of the North Adriatic eel

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https://doi.org/10.1016/j.ecss.2024.108660
Received 13 October 2023; Received in revised form 29 January 2024; Accepted 29 January 2024
Available online 9 February 2024

Abbreviations

SI	Silver Index
BL	Body Length
BW	Body Weight
Edh	Horizontal Eye Diameter
Edv	Vertical Eye Diameter
PFL	Pectoral Fin Length
HL	Head Length
PDL	Pre-Dorsal Length
BD	Horizontal Body Diameter
Bdv	Vertical Body Diameter
K	Fulton's Condition Factor
EI	Eye Index
PFI	Pectoral Fin Length Index
HI	Head Index
PDI	Pre-Dorsal Index
BCI	Body Circumference Index
GSI	Gonadosomatic Index
BWI	Body Weight Index
RAS	Recirculating Aquaculture System
FTO	Fully Transparent Oocytes
CPE	Carp Pituitary Extract
DHP	17α, 20β-dihydroxy-4-pregnen-3-one

(Mordenti et al., 2013). For the conservation efforts, the best broodstock is chosen for release and stocking programs. Hence, having a good knowledge of the spawners, knowing their maturity level and being able to recognise them, is a research objective. During the maturation process, and for the migration capacities of the individuals thereafter, the environment in which the eel is stationed also plays a principal role (Tesch, 1967). In the early stages in continental waters, eels accumulate a considerable amount of fat (lipid reserves) (Larsson et al., 1990), which will be fundamental during their migration, since once they start, their digestive system retracts and they stop feeding (Ribeiro et al., 2005). Therefore, the initial environmental conditions, like among others, the amount of food present and the local population density have a strong influence on their maturation, and on their migration success. Several studies have compared various environmental conditions and their influence on maturation. In particular, the study by Acou et al. (2003) shows how brackish environments like the *Vaccarès* lagoon are more favourable sites for growth than freshwater environments. Also, Svedäng and Wickstrom (1997) demonstrated that environmental properties could lead to large differences in lipid accumulation metabolism (i.e., growth rates). The lagoons of the North Adriatic have always been considered a favourable environment for the growth of eels because of their high and diverse food availability.

In recent years, eels from these lagoons have been used for conservation purposes, by release campaigns to encourage migration, as the LIFEEL (LIFE19 NAT/IT/000851). They are also used for artificial reproduction programs, in order to close the life cycle and obtain juveniles for use in aquaculture.

Recently, several European research groups were able to produce larvae of *A. anguilla* (Sørensen et al., 2016; Butts et al., 2016; Jéhannet et al., 2021; Politis et al., 2018; Parmeggiani et al., 2020; Sganga et al., 2022; Benini et al., 2022). While there is a long road ahead of larval weaning in captivity, the recent results are encouraging and soon could eliminate the collection of glass eels from the wild for scientific and aquaculture purposes.

The study aimed to investigate the silvering process of female eels in the North Adriatic Sea using the most representative lagoons of this area and comparing their reproduction activity at different silvering stages.

2. Materials and methods

2.1. Study area

In October–December 2022, wild female eels were caught using traditional "*lavoriero*" (downstream trap) in closed lagoons (named "*Valli*") near the sluices of the North Adriatic Sea (Italy). In these areas fisheries have been operating for centuries, taking advantage of the

Fig. 1. Sampling area of North Adriatic Sea. Position of the seven closed lagoons (*Valli*-V) investigated (source: earth.google.com).

autumn-winter migration of *A. anguilla* to the sea. The eels sampled were all wild and female eels from 7 different lagoons (Fig. 1), specifically from.

1 . ***Valli* di Comacchio** (44°36′20″ N 12°10′11″ E) (Emilia-Romagna Region): a brackish lagoon of approximately 17,000 ha. One of the most important coastal wetlands in Europe for the conservation of biodiversity and since 1988 it is protected by the institution of the Regional Park of the Po Delta of Emilia-Romagna (L.R.E.R., 1988).
2 . ***Valle* Nuova** (44°48′12″ N 12°15′16″ E) (Emilia-Romagna Region): a brackish lagoon with a spatial extension of about 2000 ha. This complex is one of the best-preserved brackish water lagoons in Emilia-Romagna from an ecological-environmental point of view and it is used for extensive aquaculture.
3 . ***Val* Bonello** (44°52′20″ N 12°23′8″ E) (Veneto Region): a brackish lagoon with an area of 50 ha. It hosts an experimental fish center ("*Centro Ittico Sperimentale*, Bonello") that works on issues about the enhancement and management of valliculture.
4 . ***Valle* San Carlo** (44°59′5″ N 12°27′21″ E) (Veneto Region): a brackish lagoon with an area of 460 ha. It receives salt water from a canal that opens eastward into the Adriatic Sea and is connected to the surrounding lagoons by canals that allow fresh water to enter directly from the Po River.
5 . ***Valle* Scanarello** (45°00′09″ N 12°22′58″ E) (Veneto Region): brackish lagoon with an area of 330 ha. *Valle* Scanarello is used for semi-extensive aquaculture and breeds the European eel.
6 . ***Valle* Ca' Pasta** (44°58′07″ N 12°19′21″ E) (Veneto Region): is an area of 240 ha. Contrary to the other lagoons of interest for this study, marine inflows are almost absent, a peculiarity that makes it a freshwater/oligo-haline lagoon.
7 . ***Val* Noghera** (45°42′30″ N 13°18′25″ E) (Friuli Venezia Giulia Region): is a brackish lagoon with an area of 1720 ha, within which there are 38 Fishing Valleys (1400 ha). The fishing valleys are managed extensively, so that water occupies on average 80% of the total surface area (Cosolo et al., 2009; Gelli, 2011). The most important fish species are European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), Mugilidae mullets and European eel (*Anguilla anguilla*) (Cosolo et al., 2009; Gelli, 2011).

2.2. Animals: morphometric parameters

All captured eels (N = 1513) were anaesthetised with a bath of clove oil (0.2 mL*L^{-1}), measured and sampled to obtain an external indicator of their maturation status (Durif et al., 2005, 2009). Morphometric parameters included: body length (**BL**, cm), body weight (**BW**, g), horizontal eye diameter (**EDh**, mm), vertical eye diameter (**EDv**, mm), pectoral fin length (**PFL**, cm), head length (**HL**, cm), pre-dorsal length (**PDL**, cm), horizontal body diameter (**BDh**, mm) and vertical body diameter (**BDv**, mm) (Fig. 2). The following indices were calculated according to the formulae below: Fulton's Condition factor (**K**), eye index (**EI**) and pectoral fin length index (**PFI**).

- **K** = $(BW{*}BL^{-3})\ {*}10^3$
- **EI** = $100{*}(((EDh + EDv)\ {*}0.25)^2\ \pi\ {*}(10{*}\ BL)^{-1})$
- ***PFI*** = $100{*}\ PFL\ BL^{-1}$

The initial stage of eels relative to the silvering process (silver index - SI) was determined according to the classification system described by Durif et al. (2005, 2009) and summarised below:

SI I-II → Stage I-II → yellow eel → resident eel,

SI III → Stage III → silver eel → pre-migrant eel,

SI IV-V → Stage IV-V → silver eel → migrant eel,

Furthermore, Head index (**HI**), Pre-Dorsal Index (**PDI**) and Body

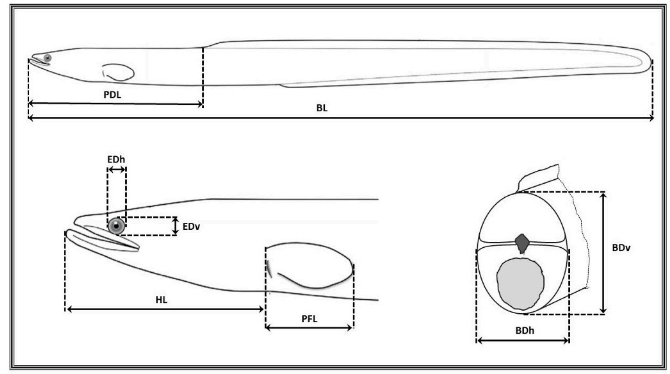

Fig. 2. Schematic illustration of measured morphometric parameters on eel. (**BL**, body length cm), (**BW**, body weight g), (**EDh**, horizontal eye diameter mm), (**EDv**, vertical eye diameter mm), (**PFL**, pectoral fin length cm), (**HL**, head length cm), (**PDL**, pre-dorsal length cm), (**BDh**, horizontal body diameter mm) (**BDv**, vertical body diameter mm).

Circumference Index (**BCI**) were calculated according to the formula below (Rye and Refstie, 1995; Watanabe et al., 2005; Emmanuele et al., 2020):

HI = $100{*}\ HL\ BL^{-1}$

PDI = $100{*}\ PDL\ BL^{-1}$

BCI= $((BDh + BDv)/2)\ \pi\ (BL)^{-1}$.

2.3. Histological analysis and age

For the evaluation of initial gonadal state, 21 females/*valle* (N = 3 residents, N = 9 pre-migrants and N = 9 migrants) were transported to the laboratories of the DIMEVET- Cesenatico. Eels were sacrificed with an overdose of 2-phenoxyethanol (800 ppm). The eels were dissected, and the gonads were removed. The gonads were weighed (**GW**, g) and the Gonadosomatic index (**GSI**) was calculated as: ***Gonadosomatic index (GSI)*** = *(GW BW^{-1}) *100*. Fragments of gonads were fixed in 10% formalin. They were then dehydrated in a solution with ethanol and soaked in paraffin. Sections of 4 μm were cut and stained with H&E (Palstra et al., 2007b).

Histological sections were evaluated with a light microscope to estimate the degree of maturation of the gonads according to Grier et al. (2009) and Gentile et al. (2022): gonad samples with oocyte maturation starting from the circumnuclear oil droplet step, i.e., oocytes in which all lipid droplets are arranged around the germinal vesicle and with a diameter around 70–80, are considered mature enough (Mordenti et al., 2013; Han et al., 2003; Pérez et al., 2011). With the data obtained, the levels of oocyte development within each silver index stage were then represented in percentages.

Age was determined using the otolithometric technique, centred on the sagittal reading, following the protocol for the measurement of the annuli given by Panfili et al. (2002).

2.4. Artificial reproduction

To compare reproductive activity, 8 females/*valle* (N = 2 residents, N = 3 pre-migrants and N = 3 migrants) were subjected to hormonally induced maturation in an artificial reproduction program.

Upon transfer to the laboratory, all animals were gradually acclimated to natural seawater over 10 days. All eels were kept in a Recirculating Aquaculture System (RAS) consisting of two fish-rearing tanks (1200 L each) and were maintained in complete darkness in seawater (salinity 31 ± 1 g*L^{-1}) at a controlled temperature of 15.5 ± 0.5 °C until gonadal maturation was complete (Mordenti et al., 2012, 2013, 2018). The animals were marked individually by inserting fish tags (FLOY TAG

Mod Floy T-Bar Anchor) and maintained in starvation for the entire duration of the trial.

The females were weekly intramuscularly injected with 5 mg*kg^{-1} BW (1st – 4th week), 15 mg*kg^{-1} BW (5th – 8th week) and 30 mg*kg^{-1} BW (9th week–final maturation) of Carp Pituitary Extract (CPE), following the originally developed protocol by Mordenti et al. (2018). When the Body Weight Index exceeded 110% (**BWI** = final BW*100/initial BW), each female was repeatedly ovary-biopsied (every 8–12 h) and the developmental stage of oocytes was evaluated by Mordenti et al. (2018). This procedure was necessary to perform the best timing of the individual final induction. Ovulation was induced by intraperitoneal 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) injection (2 mg*kg^{-1}) only when at least 50% of the oocytes were fully transparent (Fully Transparent Oocytes [**FTO**]) (Di Biase et al., 2017; Palstra et al., 2005). The developmental stage of the FTO corresponded to stage five of gamete development in European eel following Palstra et al. (2005) and to stage seven in *A. japonica* following Unuma et al. (2011). After DHP injection, each female was transferred to a new closed RAS described by Mordenti et al. (2014) and maintained for 12h with spermiating males (wild males, *sex ratio* 4M/1F) (Guarniero et al., 2020) in seawater, at a temperature of 20 ± 0.5 °C to produce a thermic shock (Mordenti et al., 2014; Guarniero et al., 2020; Dou et al., 2008), facilitating spontaneous spawning.

For all reproductive cycles, progressive gonadal maturation was observed and the number weeks to final maturation (first CPE injection until DHP injection) were registered. The gonadal maturation level was calculated in relation to the BWI achieved at the end of hormone treatment (Mordenti et al., 2012; Ohta et al., 1996) and evaluated as:

BWI<102% = no gonad maturation (NM)

BWI 102–105% = start maturation (SM)

BWI 105–110% = partial maturation (PM)

BWI >110% = full maturation (FM)

Furthermore, for each spawning event, the relative weight of spawned eggs (%BW) was calculated as a percentage of the initial BW (Mordenti et al., 2018).

Valle Noghera eels with SI II were excluded from both histological analyses and the artificial breeding programme due to logistical problems of recruitment.

Finally, the percentage variation in the **EI** of pre-migrant and migrant eels during the induced spawning program was recorded.

2.5. Statistical analysis

Morphometric (BL, BW, K, EI, PFI, HI, PDI, BCI), and GSI measurements were statistically analyzed. The data was initially checked for normality by performing a Shapiro–Wilk test. As it was not normally distributed, non-parametric statistics were applied. Statistical analysis was performed using Kruskall–Wallis test, followed by Mann–Whitney *U* test to compare data between silvering indices. The significance levels were set at $p < 0.05$ at all times, and all analyses were conducted with STATISTICA 6.0 (StatSoft Italia S. r.l.).

A histogram relating age to indices of silvering was made. Then, the normality of the dataset was tested with Shapiro-Wilk test and ANOVA statistical analysis was made. A post-hoc test (Tukey test) was carried out to verify associations between age and silvering indices. The significance level was set at $p < 0.05$ and this analysis was conducted using R (R Development Core RStudio Team, 2015; package "GAD" et al., 2018). Linear regression between age-BL and age-BW has been investigated, using the function of linear regression on R.

2.6. Ethics

All fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (DIR, 2010/63/UE). Approval for this study was obtained by the Ethics Committee of Bologna University (ID 1157).

3. Results

3.1. Morphometric parameters

The results concerning BW, BL, and K of the 1513 captured female eels from 7 lagoons of the North Adriatic Sea are shown in Table 1. In general, the percentage of migrant eels (SI IV-V) was above 50%, with occurrences of 87% in *Valle* Noghera and about 5% in *Valle* Scanarello (Table 1). Not even one migrant female eel in SI IV has been found in *Valle* Nuova. The pre-migrants (SI III) were strongly represented in *Valle* Cà Pasta and *Valle* Nuova, which represents about three-quarters of the sampling population, while for resident females (SI II), the largest sampling pool was recorded in *Valle* Scanarello (Table 1). Statistical analysis of the morphometric parameters of the sampling pools revealed an increasing trend of BW, BL, and K from resident and pre-migrant to migrant eels at silver index IV. The values of BW, BL at SI IV were statistically higher ($p < 0.05$) than the same values for eels in SI III and SI II. SI III eels were statistically larger than SI V eels ($p < 0.05$), while SI V statistically larger than SI II eels ($p < 0.05$) (Table .1). Similarly, K for migrant eels in SI IV was statistically higher ($p < 0.05$) than for resident and pre-migrant eels, and shown to be significantly higher ($p < 0.05$) than K for migrant eels in stage V.

Results of statistical analyses of EI, PFL, HI, PDI, BCI and GSI indexes are reported in Fig. 3(a–f). Significant differences were found between yellow and pre-migrant eels (EI, PFL, HI, PDI, BCI and GSI), and between pre-migrant and migrant eels (SI IV) (EI, PFL, BCI and GSI). Differences between the migrant stages (SI IV vs SI V) were found for EI, PFLI, HI, PDI and BC.

The eye indexes of SI V eels were statistically higher than for SI IV, SI III, and SI II. SI IV eels had statistically larger EI than SI III and SI II eels, and EI of SI III eels were statistically larger than the EI of SI II eels (Fig. 3). A clear decreasing eye index was observed along the decrease in silvering stages. The same trend was observed also for PFL, except for SI III eels, which group had statistically longer pectoral fins than SI IV and resident eels.

Both for HI and PDI, SI V and SI II eels had statistically higher values than SI III and SI IV eels, while there was no statistical difference between SI III, SI IV groups. SI IV eels had the highest BCI, which is statistically higher than for eels in stage V, III and II, while SI III eels have statistically higher BCI than eels in SI V and SI II.

The GSI of eels with SI V and SI IV are statistically higher than eels of SI III and SI II stages; SI III eels demonstrated statistically higher GSI than the SI II but no statistical difference was observed between the two migrant groups (SI IV and SI V).

3.2. Age

The results of the one-way ANOVA indicated significant differences between the average ages of the groups of different silvering indexes ($p < 0.0001$) (Fig. 4). Tukey post-hoc test demonstrated no difference in age between eels with silver index III and IV($p > 0.5$). Analyzing the relationship between age and weight, as well as age and length showed no direct influence of age on the weights or lengths of the eels, with $p > 0.05$ ($p = 0.122$ age-length; $p = 0.471$ age-weight) (Fig. 5 a-b). There is no clear linear regression in neither of the relations age-weight and age-length. The values of R^2 are very low and deviate in big ranges from 1 (Fig. 5 a-b). Considering the total number of individuals analyzed, eels with age below the average had a much higher weights or lengths than older eels.

Table 1
Morphometric parameters investigated in the seven North Adriatic lagoons. Data are given as the mean ± SD. Different letters (a,b,c,d) show significant differences (P < 0.05) between the different silver indeces.

LAGOON		SILVER INDEX			
COMACCHIO		***II***	***III***	***IV***	***V***
Eels	%	3.2	29.7	65.9	1.2
Body Weight (BW)	g	404.3 ± 145.7	996.45 ± 119.36	1366,94 ± 221.99	755.00 ± 175.04
Total Body Length (BL)	mm	601.1 ± 52.7	786.12 ± 43.12	848.67 ± 50.56	726.25 ± 57.17
Fulton's Condition Factor		1.79 ± 0.17	2.06 ± 0.22	2.23 ± 0.23	1.95 ± 0.17
CA' PASTA		***II***	***III***	***IV***	***V***
Eels	%	1.8	76.8	7.1	14.3
Body Weight (BW)	g	413.50 ± 8.77	747.43 ± 128.71	1020,48 ± 146.79	737.50 ± 90.23
Total Body Length (BL)	mm	648.50 ± 4.95	726.43 ± 43.26	775.13 ± 33.63	726.69 ± 35.60
Fulton's Condition Factor		1.52 ± 0.07	1.94 ± 0.17	2.18 ± 0.14	1.92 ± 0.18
VALLE NUOVA		***II***	***III***	***IV***	***V***
Eels	%	5.5	73.2	–	21.3
Body Weight (BW)	g	433.92 ± 130.46	557.09 ± 89.00	–	554.29 ± 113.00
Total Body Length (BL)	mm	620.00 ± 62.52	673.61 ± 36.20	–	670.87 ± 46.26
Fulton's Condition Factor		1.75 ± 0.22	1.81 ± 0.15	–	1.83 ± 0.23
SAN CARLO		***II***	***III***	***IV***	***V***
Eels	%	1.8	55.3	3.0	39.9
Body Weight (BW)	g	492.37 ± 57.53	616.80 ± 118.34	1143,90 ± 194.42	577.56 ± 112.70
Total Body Length (BL)	mm	668.67 ± 34.00	711.19 ± 41.61	831.80 ± 52.77	702.85 ± 39.52
Fulton's Condition Factor		1.66 ± 0.25	1.70 ± 0.17	1.99 ± 0.24	1.65 ± 0.20
BONELLO		***II***	***III***	***IV***	***V***
Eels	%	8.0	34.3	3.7	54.0
Body Weight (BW)	g	384.7 ± 92.46	544.20 ± 174.43	1157,39 ± 115.88	629.02 ± 159.10
Total Body Length (BL)	mm	630.69 ± 49.78	688.99 ± 63.71	834.75 ± 44.59	718.04 ± 64.54
Fulton's Condition Factor		1.51 ± 0.18	1.61 ± 0.22	2.00 ± 0.20	1.68 ± 0.24
NOGHERA		***II***	***III***	***IV***	***V***
Eels	%	–	13.2	1.8	85.0
Body Weight (BW)	g	–	559.34 ± 211.71	1028,64 ± 139.89	482.27 ± 170.66
Total Body Length (BL)	mm	–	680.84 ± 91.37	811.80 ± 40.61	643.66 ± 74.12
Fulton's Condition Factor		–	1.72 ± 0.29	1.92 ± 0.12	1.74 ± 0.23
SCANARELLO		***II***	***III***	***IV***	***V***
Eels	%	34.7	60	1.3	4.0
Body Weight (BW)	g	479.68 ± 81.05	626.54 ± 122.13	1023,50	422.93 ± 71.54
Total Body Length (BL)	mm	610.62 ± 41.08	668.98 ± 42.08	787.00	629.00 ± 25.00
Fulton's Condition Factor		2.11 ± 0.32	2.07 ± 0.19	2.10	1.70 ± 0.24
TOTAL		***II***	***III***	***IV***	***V***
Eels (n. 1513)	n.	71	660	256	526
	%	4.7	43.6	16.9	34.8
Body Weight (BW)	g	436.34 ± 107.78**d**	664.46 ± 201.35**b**	1345,95 ± 227.59**a**	541.35 ± 167.17**c**
Total Body Length (BL)	mm	619.33 ± 54.51**d**	706.02 ± 62.01**b**	846.75 ± 50.38**a**	673.71 ± 72.35**c**
Fulton's Condition Factor		1.82 ± 0.34**c**	1.84 ± 0.24**b**	2.21 ± 0.23**a**	1.73 ± 0.23**c**

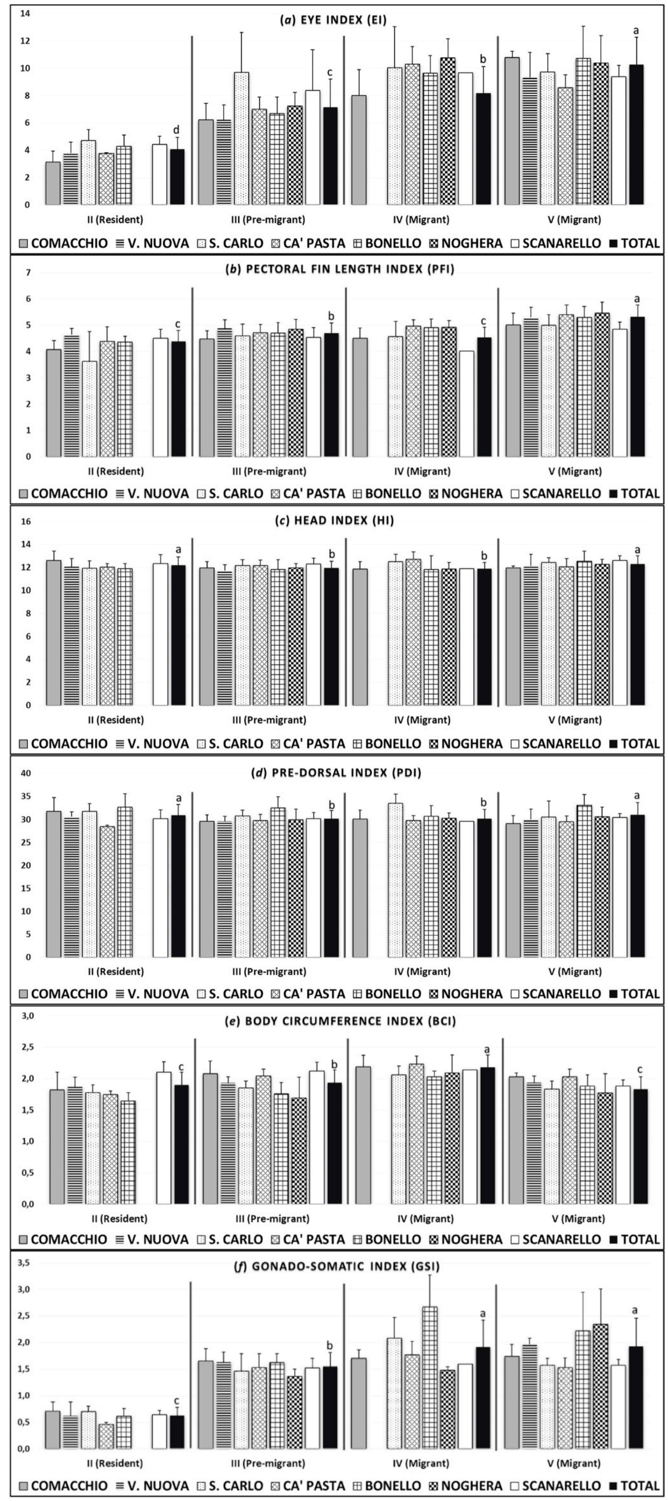

Fig. 3. Representation of the morphometric indices obtained from the respective lagoons according to the different levels of silvering. Different letters (a,b,c, d) show significant differences (P < 0.05) between the different silver indeces.

3.3. Histology and artificial reproduction

Regarding the results of histology of the oocyte maturation in the resident eels, oocytes were found in the first stages of primary growth. In the pre-migrant and migrant eels, the maturation of the oocytes is heterogeneous, as most of the eels had oocytes in different maturation stages. The trend in fact is similar; for both groups (pre-migrant,

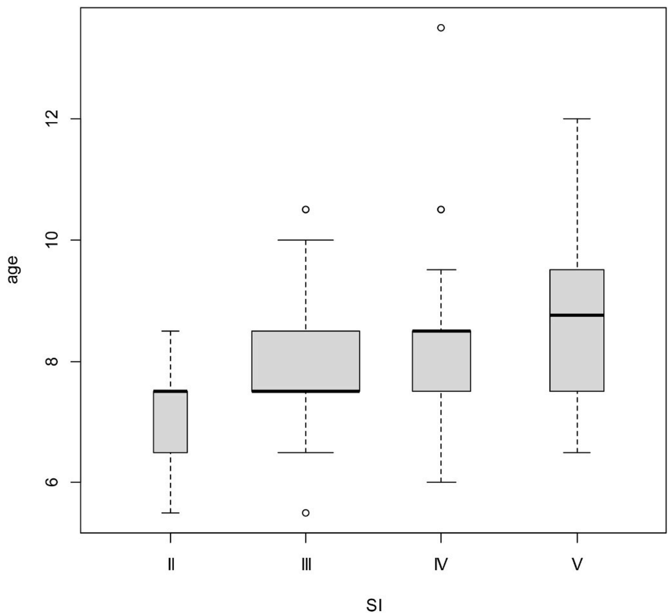

Fig. 4. Boxplot shows the relationship between age and silver indices; resident eel (SI II) have the 50% of values in a range of age 6 +/7+; pre-migrant eels (SI III) and migrant (SI IV) have the 50% of values in the same range 7+/8+ and the Tukey test shows that there is no difference (p = 0.50) between this two groups; finally, the migrant eels (SI V) have the 50% of values in a range of age 7+/9+.

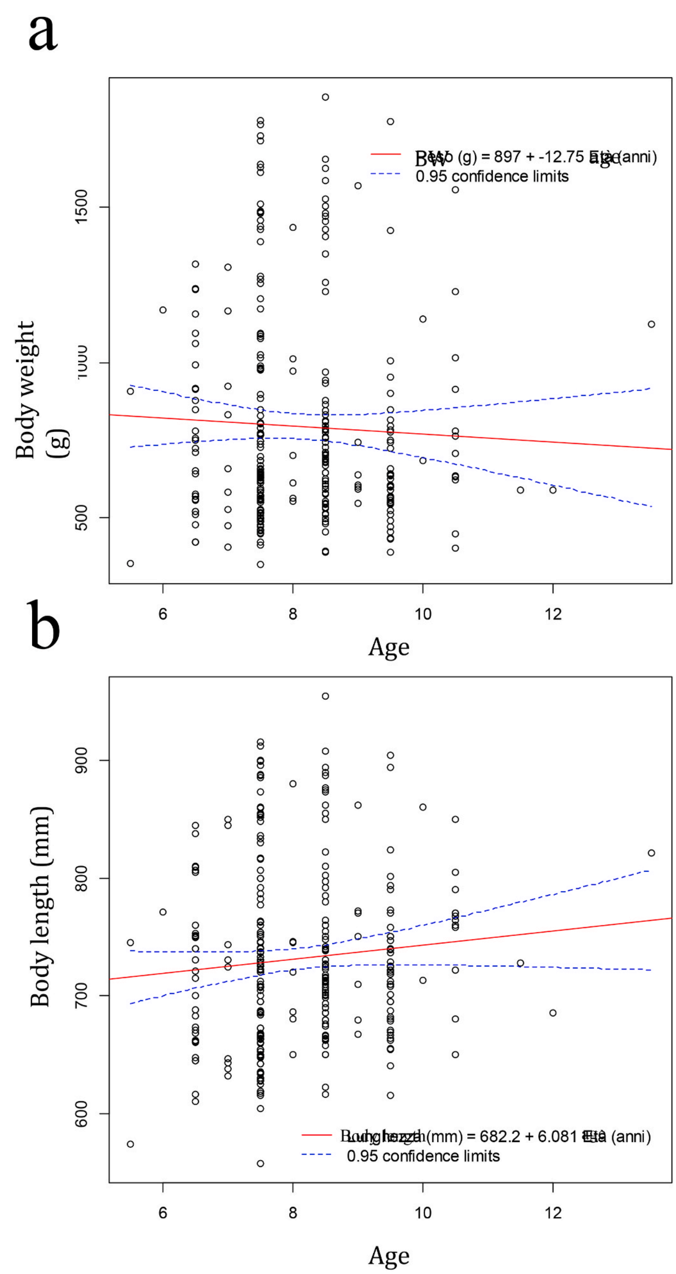

Fig. 5. **a)** Relation between age and body weight. The age has no influence on the weight of the eels confirmed by the *t*-test with p = 0.12. There is not a linear regression $R^2 = 0.004$. **b)** Relation between age and body length. The age has no influence on the length confirmed by the *t*-test with p = 0.47. There is not a linear regression $R^2 = -0.001$.

migrant) the most represented stages of oocyte development were in last three stages of primary growth and only a small percentage in the fist stage of secondary growth (i.e., the highest maturity stage found) (Fig. 6). Both pre-migrant and migrant eels show a high percentage (65% for pre-migrants and 77% for migrants) of oocytes in the most advanced stages of development.

After the hormonal induction of maturation more than 95% of the pre-migrant and migrant females reached maximum gonadal maturation (FM), while the resident females did not respond to the hormonal treatment (NM) (Table 2). Pre-migrant females mated and spawned spontaneously in the tank with a percentage of 81.0%, and 88.2% of them produced viable eggs. While migrant females mated and spawned spontaneously with a percentage of 85.7%, and 83.3% of them produced viable eggs (Table 2). The EI during the reproductive program showed an average percentage increase of 58.3% (from 7.20 ± 1.54 to 11.41 ± 2.74) for the originally pre-migrant females, while for the migrant eels this percentage increase was only 29.6% (from 9.30 ± 1.90 to 12.05 ± 2.91).

4. Discussion

The process of silvering in eels was first studied by Pankhurst (1982), Boëtius and Boëtius (1980), Fontaine (1994) and then extended and deepened by Durif et al. (2005, 2009) in Atlantic eels. Indeed, the original simple distinction between yellow and silver eels does not consider the complex preparatory phase (the silvering process), during which the animal undergoes morphological and physiological transformations in preparation for the long migration to their mating and spawning area (Durif et al., 2005). Feunteun et al. (2000) classified eels into three stages: yellow, silver and yellow/silver, but these stages were only based on external and visual variables (skin colour and visibility of the lateral line), but no physiological or morphological evaluation was made to define the differences between the different stages. The silver index method developed by Durif et al. (2005), involves classifying females into five stages and subdividing them into resident, pre-migrant and migrant eels. In addition of being well defined, it also represents a way to standardise observations between eel populations from different environments and geographic areas.

Despite the morphometric differences that characterized the eels of each *valle*, the trend observed was in accordance with the observed trend by Durif et al. (2005, 2009) of European eel connected by their migration route to the Eastern Atlantic Ocean (Atlantic eel), sampled in different habitats in France. This trend is described as a general increase in weight, length, and K-index from the stages of the resident eels up to migrating eels in stage IV, then a reduction in these parameters once the maximum level of silvering (SI V) is reached. If, however, measuring the weight changes in percentages within each stage, a different trend can be observed. Among the eels sampled in the Nord Adriatic lagoons, the greatest growth rate was observed during the transition from premigrating to migrating eels (SI III to SI IV = 102%), but Durif et al.(2005,

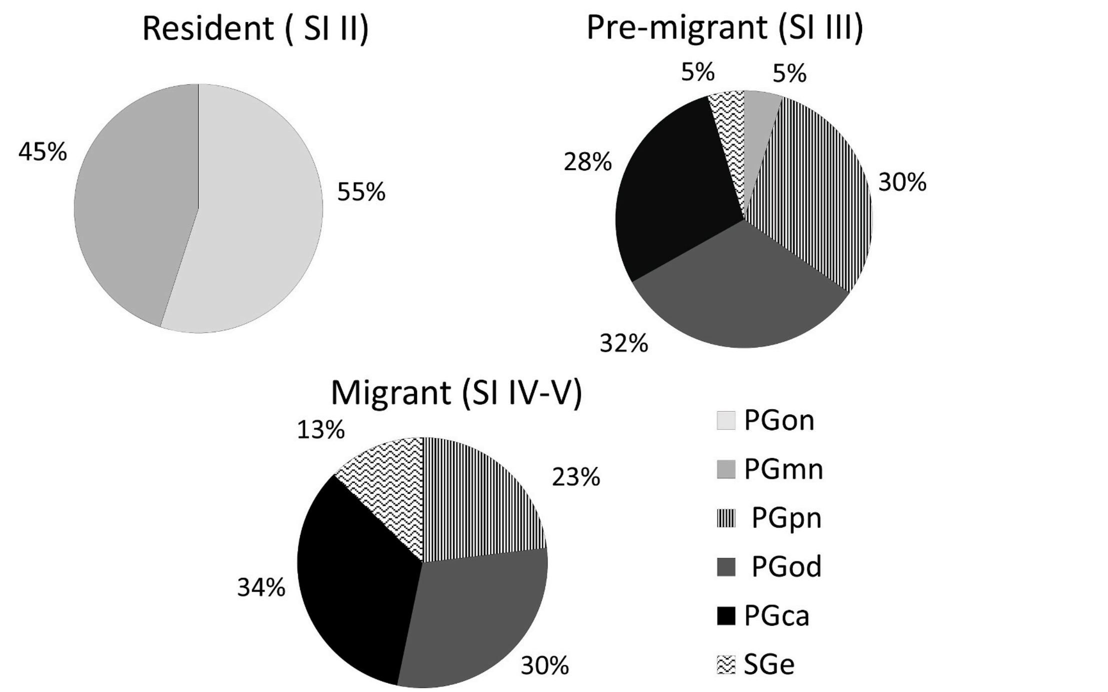

Fig. 6. Percentage distribution of gonadal maturation stages of eels in three different stages (resident, pre-migrant, and migrant). PGon = single nucleolus stage, PGmn = multiple nucleolus stage, PGpn = perinuclear stage, PGod = circumnuclear oil drop stage, PGca = cortical stage, SGe = early secondary growth stage.

2009) observed the greatest growth rate earlier, during the transition from resident to pre-migrating eels (SI-II to SI-III = 107%). Therefore, the Adriatic eels entering in SI IV stage likely have not yet stopped feeding. To confirm this, Mordenti et al. (2012) observed that silver eels kept in captivity undergoing an artificial breeding program continued to feed. In contrast, a comparison of stage IV and V eels shows a much greater reduction in weight in case of the Adriatic eels (SI-IV to SI–V = −148%) than in the Atlantic eels (SI-IV to SI–V = −86%) (Durif et al., 2005, 2009). Probably a part of the sampled eels was blocked by the *lavorieri,* (downstream traps), which have a catching efficiency close to 100% (Aschonitis et al., 2017). These traps can effectively prevent their spontaneous migration to the sea, forcing them to stay for quite some time (even years) inside the *valli* although their food tract has already started degenerating. In fact, according to Durif et al. (2009), eels stranded or disturbed during migration (by dams or river obstructions) can resume a sedentary life and wait another year to continue their migration.

North Adriatic eels also showed a higher average weight and length in all 4 stages comparing to Atlantic eels. The strong presence of relatively large eels is certainly derived from the favourable environmental and trophic situation of the entire North Adriatic lagoon area. In this regard, numerous studies highlight the abundance and variety of prey [larvae of insects, prey snails, mussels, crustaceans (*Crangon crangon* and *Palaemon* sp.) and fish (*Atherina boyeri, Engraulis encrasicolus, Aphanius fasciatus, Pomatoschiustus* sp.)] available to eels in the brackish lagoon areas north and south of the Po River delta (De Leo and Gatto, 1995; Mordenti et al., 2013, 2018, 2016; Emmanuele et al., 2020). The good initial conditions are also confirmed by the resident eels, which showed significantly higher average weight and length than those surveyed near the Atlantic (Durif et al., 2009). Also confirming the excellent trophic level in this region were the eels of Valle Ca' Pasta, a freshwater lagoon that represents a completely different environment. However, the eels in Valle Ca' Pasta had an overlapping growth rate with the eels sampled from the other, brackish lagoons studied. This seems to contrast with other observations reporting that European eel grew faster in brackish than in fresh-water environments (Fernandez-Delgado et al., 1989; Panfili et al., 1994; Melià et al., 2006).

The large size of Adriatic eels is characteristic of those found in the eastern Mediterranean. Moreover, when comparing our results with the results from Vistonis Lake (Northern Aegean Sea, Greece) (MacNamara et al., 2014), there is a complete overlap in size characteristics. There appears to be a positive correlation between animal size and the distance from the breeding site. Vøllestad (1992) and Durif and Elie (2008) also observed a positive correlation between length during the silvering transformations and the distance from the spawning site. Durif et al. (2006) showed that eels over 70 cm have a higher gonad weight/body length ratio, which represents a benefit in terms of fecundity, and Larsson et al. (1990) showed that large eels with a "critical fat mass" are favored in the process of silvering. It is thus presumable that the reproductive potential of females in the Northern Adriatic is very high. This hypothesis is partly reinforced also by a study of Tesch (1980), according to whom the migratory phase depends more upon size than age.

The good growth and excellent nutritional status of the eels surveyed are also confirmed by the values recorded for age, which was found to be within a very narrow range (from 7 to 7+ to 8-8+) for about two-thirds of the different sampling pools. The females of the Northern Adriatic were found to be much younger than other surveyed eels in the Atlantic and Northern Europe (Vøllestad et al., 1986; Svedäng et al., 1996; van den Thillart et al., 2009), while they are in accordance with other studies carried out on eels in the eastern Mediterranean (Mordenti et al., 2013; Emmanuele et al., 2020; MacNamara et al., 2014). Furthermore, if we compare the age classes of the animals with their silver index, the results show that resident eels are on average were much younger than migrants, while the most represented age groups in the pre-migrant group (SI III) (7-7+ and 8-8+ age groups) are more similar with those recorded for migrant stages (SI-IV and SI–V). The observation that a high number of eels with SI-III characterized by the same average age as the migrant females, caught in the migration period leads to two conclusions. These results confirm the observation of Palstra and Planas (2011) about the transition from pre-migrant to migrant to be a fast process, and individual animals can undergo the silvering stages during the 3–4 months of the same migration period. The other conclusion is that the Adriatic pre-migrants, although not fully formed from a morphological point of

Table 2

Zootechnical results from artificial reproduction trials. Data are given as the mean ± SD. ***Gonad maturation*** calculated in relation to the BWI achieved at the end of treatment: *GML*(Gonad Maturation Level)* = no gonad maturation (NM), start maturation (SM), partially maturation (PM), fully maturation (FM); *Week* (number of weeks to reach gonad maturation); ***Reproduction***: *Yes* = successful reproduction, *No* = unsuccessful reproduction; ***Spawned eggs:*** *Yes* = successful, *No* = unsuccessful, *%BW* (the relative weight of spawned eggs was calculated as a percentage of the initial BW).

LAGOON	GONAD MATURATION		REPRODUCTION	SPAWNED EGGS	
	GML*(n)	Week	Yes/No – n./Total	Yes/No – n./Total	%/BW
Comacchio					
Resident (SI II)	NM (2)	–	N	N	–
Pre-migrant (SI III)	FM (3)	22.33 ± 2.08	Y-3/3	Y- 2/3	35.45 ± 4.17
Migrant (SI IV-V)	PM (1) FM (2)	20.67 ± 1.15	Y-2/3	Y- 2/2	39.79 ± 6.21
Cà Pasta					
Resident (SI II)	NM (2)	–	–	–	–
Pre-migrant (SI III)	FM (3)	19.67 ± 0.58	Y-2/3	Y-2/3	40.1 ± 1.41
Migrant (SI IV-V)	FM (3)	19.33 ± 0.58	Y-3/3	Y-3/3	35.44 ± 3.53
Valle Nuova					
Resident (SI II)	NM (2)	–	N	N	–
Pre-migrant (SI III)	FM (3)	21.67 ± 2.08	Y-3/3	Y-3/3	41.77 ± 3.56
Migrant (SI IV-V)	FM (3)	21.33 ± 1.53	Y-3/3	Y-2/3	42.45 ± 3.61
San Carlo					
Resident (SI II)	NM (2)	–	N	N	–
Pre-migrant (SI III)	FM (3)	20.33 ± 2.08	Y-2/3	Y-1/2	22.5
Migrant (SI IV-V)	FM (3)	20.67 ± 2.08	Y-2/3	Y-1/2	44.3
Bonello					
Resident (SI II)	NM (2)	–	N	N	–
Pre-migrant (SI III)	FM (3)	17.67 ± 0.58	Y-2/3	Y-2/2	35.42 ± 5.10
Migrant (SI IV-V)	FM (3)	18.33 ± 0.58	Y-3/3	Y-3/3	36.33 ± 7.98
Noghera					
Resident (SI II)	–	–	N	N	–
Pre-migrant (SI III)	FM (3)	21.00 ± 3.00	Y-3/3	Y-3/3	36.63 ± 4.99
Migrant (SI IV-V)	FM (3)	20.33 ± 2.31	Y-2/3	Y-2/2	40.78 ± 2.18
Scanarello					
Resident (SI II)	NM (2)	–	N	N	–
Pre-migrant (SI III)	PM (1) FM (2)	21.33 ± 1.53	Y-2/3	Y-2/2	39.04 ± 0.87
Migrant (SI IV-V)	FM (3)	20.33 ± 0.58	Y-3/3	Y-2/3	33.90 ± 5.18
TOTAL					
Resident (SI II)	***NM (12)***	**-**	***N***	***N***	**-**
Pre-migrant (SI III)	***PM (1) FM (20)***	***20.57 ± 2.16***	***Y-17/21***	***15/17***	***37.18 ± 5.59***
Migrant (SI IV-V)	***PM (1) FM (20)***	***20.14 ± 1.53***	***Y-18/21***	***15/18***	***38.23 ± 5.33***

view, nevertheless already have an active migratory instinct. The delicate transition from resident to pre-migratory phase probably takes place in a single growing season (spring-summer) and lasts until the autumn period (Fontaine, 1994; Casalini et al., 2022). Confirming this, Durif et al. (2009) pointed out that the July–August period is clearly a crucial period for eels to undergo the silvering process without, however, highlighting whether this process can extend into the autumn months.

Concerning age-BL and age-BW relationships, our results showed no statistically significant correlation for both relationships, and it is evident that the values are not distributed along precise curves (Fig. 5(a) and b). The heterogeneity of the values and their distribution without a clear pattern suggests that age is not a defining factor for understanding the degree of maturation.

The survey of the silvering index showed a low number of resident eels (SI II) caught, a good presence of pre-migrant eels and a percentage of migrant eels above 50 %. The small number of yellow eels sampled (4.7%) was conceivable because the catches were made during the most favourable period for migration. Their presence in the *lavorieri* close to the open sea is probably occasional, due solely to behavioral instincts dictated by imitating the movement of the silver eels and not a true migratory impulse. Moreover, most of the yellow eels came from *Valle* Scanarello (46.5%), the only *valle* in which there was an integrated an inert diet and therefore characterized by eels with less active migration (van den Thillart et al., 2009). In contrast, the high number of eels caught in the *lavoriero* (over 40%) in the pre-migratory stage (SI III), leads to the assumption that the migratory instinct in the eels of the investigated areas is already present when the transformations associated with the silvering process is not yet completed.

The trend observed in the respective stages of silvering in relation to PFI and EI is in accordance with that observed by Durif et al. (2005, 2009). However, in case of the Atlantic eels, during the transition from pre-migrant to migrant stages, an increase of more than 40% in eye size can be observed (from 7.6 in SI-III to 10.8 in SI-IV), which is a way bigger increase compared to the Adriatic eels investigated, where the increase in EI stops at 15% (from 7.14 in F III to 8.19 in F IV). As already observed by Emmanuele et al. (2020), for eels from the same area as this study, this aspect can be explained by the shallow waters which describes the entire lagoon and marine area of the Northern Adriatic, and therefore in the initial phase of migration, the need to capture light, and thus the need to maximize eye dilation is not yet as strong as in case of other regions. This could also justify why in Atlantic eels EI reaches its maximum value in SI IV while in Adriatic eels EI continues to increase up to SI V. Finally, our studies confirm what has been observed by other researchers as well (Pankhurst, 1982; Durif et al., 2005; Durif et al., 2009; van den Thillart et al., 2009), i.e., that the eye index remains by far the value that best expresses the transition from resident to migrating eel.

As far as HI and PDI are concerned, the results show an overlapping trend with a significant reduction in values during the transition from resident to pre-migrant eel (from SI II to SI III) and a significant increase in these indices within migrating eel stages during the attainment of the maximum silvering stage (from SI IV to SI V). While the results confirm what has been observed by other researchers (Durif et al., 2005, 2009; van den Thillart et al., 2009; Rousseau et al., 2009), i.e., the morphological changes of the silvering eels are pointing towards to obtain better hydrodynamics for the migration, with elongation and thinning of the skull. This study shows that the morphological changes to obtain better hydrodynamic characteristics does not only concern the cephalic portion, but seems to consider also morphological changes of the anterior part of the body, as PDI was statistically lower for eels in stage III and IV, in stages of full downstream migration when the growth of the animal has stopped. This would also explain the reduction in HI and PDI values observed in the transition from SI II to SI III, as from this phase change, the eel is seeking maximization of the weight parameter, an aspect also confirmed by the increase in BCI. The same reason can be argued to justify the “brachymorphic” appearance already observed in female eels from the *Valle* Comacchio by Emmanuele et al. (2020).

Another very interesting aspect concerns the GSI found in the Adriatic eels. If in fact resident and migrating eels showed similar GSI values to the Atlantic populations, the values found in the pre-migrant Adriatic eels showed indices that were practically double those of the Atlantic eels investigated by Durif et al. (2005, 2009) (1.55 ± 0.25 vs 0.88 ± 24 respectively). In addition, histological analysis of the gonads of SI III eels showed high levels of oocyte development and gametogenesis activity, which overlapped of eels in migratory stages. An identical level of oocyte development in pre-migratory Adriatic eels was also recorded by Gentile et al. (2022) and Casalini et al. (2022). The same authors had also already observed different levels of gonadal maturation compared to Atlantic eel populations.

The morphological and gonadal survey values, combined with the age of the animals, suggest that the pre-migrant female eels, present in the lagoon areas of the North Adriatic are ready for migration. The EI of the pre-migrants itself showed an even higher percentual increase during the artificial reproduction program than for samples of any other more advanced silvering stages. An index, which is one of the most commonly used to assess sexual maturity, as direct correlation between eye size and gonadal development in female eels is now widely proven (Pankhurst, 1982; Feunteun et al., 2000; Durif et al., 2005, 2009; van Ginneken et al., 2007).

Zootechnical results from artificial reproduction trials also support this theory. Resident eels usually do not respond well to the hormonal stimulation programs, demonstrating that they are not ready to migrate. Pre-migrant eels from the different *valli* areas near the Nord Adriatic Sea showed reproductive results that were completely overlapping with the migrant eels subjected to the same reproductive program. Both in terms of time to reach maximum gonadal maturation and in egg production numbers. The SI III females all reached full gonadal maturity, 81% of them spawned naturally in the tank without the usage of striping technique, and 88% of them produced egg quantity around 37% of their BW. These values are completely overlap with the values of other artificial reproduction trials on SI IV and SI V eels from the same Adriatic areas (Mordenti et al., 2013, 2018; Di Biase et al., 2016). Reinforcing this theory, a study by Okamura et al. (2008) on *A. japonica* showed that in an artificial breeding program, females with lower levels of silvering were unable to respond well to sexual maturation inducing hormonal treatment. In addition, the time required (number of weeks of the hormonal treatment) to reach the final oocyte maturation phase, when injected with SPE directly, for eels, it relates to the developmental stage of the gonad before hormonal treatments (Ijiri et al., 1998). The maturation status just before hormonal treatments, which is often assessed through the silvering scale developed by Durif et al. (2005), is an important factor for the success of the subsequent induced maturation and spawning. In case the local population differs from the European eel, which are migrating through the Eastern Atlantic shores, the scale might need calibration to be used for artificial reproduction, stock management, and for release programs, required by the European Regulation (EC, 2007).

For the reasons presented by this and previous studies (Mordenti et al., 2023), the authors highlight that the developmental scale proposed by Durif et al. (2005) seems not always applicable to the eels migrating through the Northern Adriatic. In particular, stage V females investigated for this study, considered migrants, are the most disadvantaged to undertake migration. More specifically, SI V eels undergo severe weight loss and even regression in gonadal maturation, using them for release and re-stocking actions could be counterproductive, considering the smaller probability of the successful migration. On the contrary, the excellent results of the artificial reproduction program suggest that the eels at silvering stage III can be used efficiently for this type of actions.

To assess the morphological and gonadal development of European female eels in the Nord Adriatic region, the model adopted by Pankhurst (1982), in which eye indicators alone can be used to define the maturity of females in absolute and objective terms, seems to be more practical and simply applicable. This study, carried out on over 1500 females, confirmed EI values that can guarantee with a high percentage of accuracy the distinction between a female eel classifiable as “sexually immature adults” and a female eel, classifiable as “sexually maturing adults”, ready for ocean migration. As a result of a very high size discrepancy, which was observed between resident eels (EI 4.08 ± 0.85 mm) and pre-migrant eels with SI III (EI 7.14 ± 1.83 mm), the distinction can be easily assessed for the local populations of the different inland waters connected to the Nord Adriatic. Already Fontaine (1994) had noted this clear deviation in EI between resident eels (from 2.97 to 5.70) and migrating eels (from 5.58 to 11.4). The analysis of the EI in the Adriatic females revealed another interesting aspect. Considering only one specific parameter, the horizontal eye diameter (EDh - mm), the results of this study demonstrate a greater difference of EDh between eels with SI II (EDh 5.86 ± 0.52 mm) and SI III (EDh 7.85 ± 0.83 mm) that was greater than that recorded in the EI. This would mean that by measuring only one parameter (EDh) we could identify a female ready for migration with a margin of safety equal to the EI. Confirming this, Casalini et al. (2023) developed a user-friendly protocol (app) to identify the maturity of an eel by measuring Edh.

5. Conclusion

The study aimed to investigate the morphological and reproductive characteristics of female eels in the North Adriatic Sea. The results showed how the North Adriatic could present environmental conditions that favor the fast development of female European eel with a noticeable reproductive potential to be used for stock management. In the seven populations investigated, the growth phase (SI II) and migration phase (SI IV and V) were well defined, while the pre-migration phase (SI III) was not clear. While morphometric parameters seem to show eels at the beginning of the morphologic transformations of the silvering process, the results recorded for GSI, oocyte development levels and reproductive performance in captivity are statistically the same as for stage IV and V eels, which suggest that stage III eels also have all the prerequisites to be considered ready to migrate and to use them for artificial reproduction programs and release campaigns.

Funding

This work was funded by the “IMORDENTI 16APIS/2016” given to Dr O. Mordenti, by the RFO Grants of the University of Bologna to Dr O. Mordenti.

CRediT authorship contribution statement

Antonio Casalini: Writing – original draft, Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Laura Gentile:** Writing – original draft, Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Pietro Emmanuele:** Writing – review & editing, Methodology, Investigation, Data curation. **Alberto Elmi:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Oliviero Mordenti:** Writing – original draft, Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

This study was supported by the LIFEEL project (LIFE19 NAT/IT/000851).

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