Chapter 2
Seasonal Evolution and Individual Differences in Silvering Eels from Different Locations

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2.1 Introduction

Silvering is a requirement for downstream migration and reproduction. It marks the end of the growth phase and the onset of sexual maturation. This true metamorphosis involves a number of different physiological functions (osmoregulatory, reproductive), which prepare the eel for the long return trip to the Sargasso Sea. Unlike smoltification in salmonids, silvering of eels is largely unpredictable. It occurs at various ages (females: 4–20 years; males 2–15 years) and sizes (body length of females: 50–100 cm; males: 35–46 cm) (Tesch 2003). It is most common when studying eels, to separate individuals into two groups, yellow (resident) and silver (presumably migrant), and to compare the physiological profiles between the two. Basic knowledge was obtained in this way and we will first review what is known about these two stages.

Because of the difficulty of getting individuals while they are in the process of metamorphosing, little is known about the dynamics of the silvering process. Here we present new information on the triggers, duration and succession of events up to

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the silver migratory stage. The transitional stages – between the yellow (resident) stage and the silver (migrating) stage – were characterized through exhaustive sampling of both resident and migratory eels. New aspects concerning the endocrinology of the silvering process will be given based on seasonal sampling of eels undergoing metamorphosis. These results will also bring new insights on the triggers and timing of silvering.

2.2 Literature Review

2.2.1 Characteristics of Silver Eel

2.2.1.1 External Changes

The first obvious change in the eel is the colour modification. Migrating eels display counter-shading similar to pelagic fishes and marine mammals. The belly turns silvery white, while the sides and back, initially yellow or dark green, become a dark bronze colour. This colour change is viewed as an adaptation to the pelagic environment. As in salmonids this change occurs before the fish actually enters the marine environment. Other distinctive features of silver eels are their enlarged eyes, their elongated pectoral fins, which become black and the increased thickness of their skin.

2.2.1.2 Energy Stores

Migratory eels must have the necessary energy stores to accomplish their long spawning migration. Lipids are the main source of energy in fishes, especially migratory fishes. In eels, accumulation of fat seems to occur during silvering, since in yellow eels energy stores are mainly glycogen (Barni et al. 1985). Fat stores increase from 8% to 28% between the yellow and silver stage (Bergersen and Klemetsen 1988; Larsson et al. 1990). A small portion of the fat will be used for gonad development while the majority will be used for swimming. These lipids are thus in direct contact with the muscles (Fontaine 1975; Pankhurst 1982a). Fat is also stored under the skin and in the liver. Larsson et al. (1990) hypothesized that a certain amount of fat stores (28%) are a requirement for eels to silver. However many migratory eels display lower percentages (Svedäng and Wickström 1997).

2.2.1.3 Sensory Organs

The size and structure of the eye change during silvering. The volume and consequently the surface of the eye are considerably increased (Bertin 1951; Stramke 1972; Pankhurst 1982b). This makes the eye of silver eels more efficient in absorbing light

than that of yellow eels (Carlisle and Denton 1959). In addition, the number of rods increases and cones appear to decrease since the retinal surface area increases (Pankhurst 1982b). Lack of cone cells is common in deep-sea fish where color vision is unnecessary (Locket 1975). Before eels even reach the sea, their retinal pigments shift from porphyrin and rhodopsin to chrysopsin which is for scotopic vision (Carlisle and Denton 1959; Es-Souni and Ali 1986). Maximal absorption of the pigment is shifted towards short wave lengths as it is in deep-sea fishes.

The lateral line is an important sensory organ used by fishes to detect currents in the close environment to avoid obstacles, capture prey or avoid predators, and for schooling. During silvering, the lateral line becomes visible and sensory cells increase (Zacchei and Tavolaro 1988).

Olfaction is particularly well developed in eels. However, Pankhurst and Lythgoe (1983) and Sorensen and Pankhurst (1988) showed that olfactory cells and associated mucus cells degenerate in male and female eels after hormonally induced sexual maturation. This suggests that olfaction will be less important during the marine migratory phase of the life cycle than during the freshwater phase when eels are still feeding. However, later in the life cycle, during sexual maturation, development of sensitivity to sexual pheromones is likely to occur and to be involved in the expression of the spawning behaviour.

2.2.1.4 Alimentary Tract

Silvering eels stop feeding and this continues until the end of their life cycle. This occurs in August according to Bertin (1951). The alimentary tract degenerates, and this is further increased during hormonally induced sexual maturation. However, it still plays a role in osmoregulation (Chapter 6). First, the intestine becomes shorter, and then the thickness of the wall decreases. This partial degeneration is not merely a consequence of fasting but a true degeneration (Pankhurst and Sorensen 1984). This state is reversible if the eel starts feeding again, even after complete sexual maturation (Dollerup and Graver 1985; Le Belle and Fontaine 1987).

2.2.1.5 Swim Bladder

Most fish tissues are denser than water and therefore fishes tend to "sink". The swim bladder of fishes decreases density by secretion and re-absorption of gas. It allows the fish to adjust its buoyancy. Silver eels can secrete more gas than yellow eels. This is due to a considerable development of the capillaries of the *rete mirabilis* which increase in diameter and in length (Kleckner and Krueger 1981; Yamada et al. 2001). Guanine coats the wall of the swim bladder which becomes thicker, thus reducing loss of gas by diffusion (Kleckner 1980). These modifications are considered as an adaptation to life in the deep sea (see chapter 5).

2.2.1.6   The Osmoregulatory System

The eel is euryhaline, meaning it can withstand rapid and large changes in salinity. The immediate passage from freshwater to saltwater causes a loss of water and therefore increases ion concentration in the blood. Osmotic balance is achieved by absorption of water by the alimentary tract. The intestine, the kidneys, and the gills eliminate ions. Mucus is more abundant and the thickness of the skin increases to reduce loss of water (Fontaine 1975).

In contrast with salmonids, the eel adapts quickly to saltwater whatever the stage. Yellow eels can be found both in fresh- and saltwater, so changes in osmoregulatory function are less important. Nevertheless, yellow eels seem to adapt more slowly to saltwater than migratory eels according to Boucher-Firly and Fontaine (1933), but this will be further developed in Chapter 6. As long as silver eels stay in freshwater, they will undergo a progressive demineralization (Fontaine 1975; Dutil et al. 1987). According to Fontaine (1985) this state could stimulate silver eels to migrate and leave freshwater.

2.2.1.7   Gonads

Eels become sexually differentiated when they reach 14 to 35 cm in length (Bienarz et al. 1981; Colombo et al. 1984). In captivity, gonads were differentiated after 2 years (Beullens et al. 1997). In male eels, differentiation occurs at the same time as silvering while gonads of female differentiate well before (Colombo et al. 1984; Durif et al. 2005).

In females, the gonad weight increases during silvering. Nevertheless, the gonadosomatic index (GSI) rarely goes above 1.5%, while it can reach 50% after hormonally induced sexual maturation. The gonadotropic axis is blocked as long as the eel remains in fresh- or coastal water. It is surmised that the eel naturally becomes sexually mature only in the open ocean (although this has never been observed) and under the influence of certain, yet unknown, environmental factors.

2.2.2   Hormonal Control

Silvering is considered to be a secondary metamorphosis because during this phase the eel undergoes many morphological and physiological modifications. The neuroendocrine system regulates these modifications. Analogies with the parr-smolt transformation or smoltification of diadromous salmonids are often made, since these both correspond to a pre-adaptation to the marine environment. Changes in skin colour are similar in smolts and silver eels have counter-shading typical of marine species. In smolts, the most important changes concern the osmoregulatory system. By analogy, the first studies on endocrinology of silvering focused on these hormones and their role in the osmoregulatory changes. However, one must bear in

mind that smoltification relates to juvenile fish (smolts) while silvering marks the onset of sexual maturation and the most important changes in silvering eels relate to the reproductive system.

Modifications in the activity of ionic transport in gills have been linked with production of cortisol and thyroid hormones have been implicated in this. According to histology, the thyroid gland is more active in silver eels than in yellow eels (Callamand and Fontaine 1942). The production of thyroid hormones induces hyper-locomotor activity. One can read many anecdotes on the agitation of eels at the onset of migration. Captive eels will try to escape and this particular behaviour has been used by fishermen to predict on which nights migratory runs of eels will occur (Fontaine 1975). Hyperactivity caused by hyperthyroidism could even cause them to leave the water if there is no outlet, as occurs in aquatic amphibian larvae before their adult metamorphosis (Fontaine 1975). Han et al. (2004) found that transcription of the beta subunit of TSH (thyroid stimulating hormone) increases along with serum thyroxine levels during silvering of the Japanese eel, *Anguilla japonica*. However in the European eel, recent results have shown that thyrotropin mRNA levels did not change nor did serum thyroid hormone increase during silvering (Aroua et al. 2005). Aroua et al. (2005) did not find any effect of T4 treatments on yellow eels and they concluded that the thyrotropic axis is not or only moderately involved in silvering.

Adrenocorticotropic hormone (ACTH) secreted by the pituitary gland stimulates the adrenal gland and boosts the synthesis of corticosteroids. Leloup-Hâtey (1964) showed that adrenal cells increase in numbers and serum cortisol level increases during silvering. It has been shown in the North American eel, *Anguilla rostrata*, that cortisol induces a rise in the activity of Na^+, K^+ ATPase of gills and intestine as well as silvery hues in yellow eels (Epstein et al. 1971). Utida et al. (1972) showed that water absorption in the gut was increased in cortisol-treated freshwater eels. Finally, cortisol probably has a role in lipid breakdown in fasting silver eels (Fontaine 1975).

The changes described previously remain slight in comparison with changes related to the beginning of sexual maturation. Silvering marks the onset of sexual maturation. Among all vertebrates, the development and activity of gonads are under the positive control of gonadotropins (GTH: LH: luteinising hormone and FSH = follicular-stimulating hormone) secreted by the pituitary. Gonads will produce gametes and steroid hormones in response to GTH stimulation. It is at this gonadal level that sexual maturation can be artificially induced by injecting carp or salmon pituitary extracts in females and hCG (human chorionic gonadotropins). Steroids will in turn stimulate target organs involved in reproduction. This will allow yolk deposition (vitellogenesis) in oocytes. Vitellogenin protein is synthesized by the liver under the effect of estradiol and incorporated in the oocyte under the control of GTH. Only silver eels have hepatocytes capable of being stimulated for this synthesis via estradiol receptors although large extraphysiological doses of estradiol can stimulate vitellogenin synthesis in yellow female eels as well as in male eels (Burzawa-Gérard et al. 1994). Silver eels also have the structures necessary for endocytosis of vitellogenin.

Gonadotropic function is totally inactive in yellow eels, while it shows some activity in silver eels (Dufour et al. 1983a, b; Durif et al. 2005). The brain controls the production of pituitary LH (luteinising hormone) via GnRH (gonadotropin releasing hormone) produced by neurosecretory cells located in the hypothalamus. The status of these neurons (activation) can depend on the developmental stage of the animal (age, growth, and metabolism) as well as on environmental factors. The blockade of sexual maturation in the silver eel is due to an insufficient production of GnRH and to a dopaminergic inhibition that prevents the production of LH (Dufour et al. 1991). At the yellow stage this blockade is reinforced since gonads do not respond to pituitary extract (Lopez and Fontaine 1990). Combined treatments of GnRH agonist and dopamine receptor antagonist resulted in a significant increase in pituitary LH as well as increased locomotor activity of eels (Dufour et al. 1988).

Growth hormone, another pituitary hormone, stimulates growth and cell reproduction in animals. In addition, it acts on several physiological processes, including reproduction, metabolism and osmoregulation (Evans 1993). GH also stimulates estradiol receptors in the liver (Messaouri et al. 1991) and potentiates estradiol-induced vitellogenin synthesis (Peyon et al. 1996). GH also controls the secretion of IGF-I (Insulin-like Growth Factor 1) by the liver in teleosts as in other vertebrates. Huang et al. (1998) showed that IGF-I may exert a negative feedback on growth by inhibiting pituitary GH secretion but a stimulatory effect on reproduction by activating LH production. Estradiol and testosterone then amplify the activity of the gonadotropic axis by stimulating the production of LH while inhibiting GH.

It is not known what triggers silvering in eels. In vertebrates, sexual maturation occurs when individuals have reached a certain age and size and accumulated enough energy stores to ensure the success or reproduction. In eels, it has been suggested that there is a "critical fat mass" for triggering silvering (Larsson et al. 1990); although other studies did not find any link between fat content and silvering (Svedäng and Wickström 1997).

Huang et al. (2001) have shown that in vivo injections of cortisol and testosterone have a stimulatory effect on LH synthesis. Yellow eels that received this treatment also showed silver coloring, increase of retinal surface area, increase in liver weight, and regression of the gut. Similar results were obtained with 17α-methyltestosterone (11-KT) implants on *Anguilla australis* (Rohr et al. 2001). Six weeks after implantation, treated yellow eels displayed increased eye surface area, darkening of the pectoral fins, thickening of the skin and development of the liver and gonads. It appears that 11-KT is capable of inducing silvering. Recent data highlighted the possible roles of gonadotropins (FSH then LH) in the control of silvering and are reported in Chapter 3 of this book.

2.2.3 Length and Age at Migration

Length and age at migration are extremely variable in female eels. Differences in male and female growth rates and strategies have often been discussed but it is not clear why certain eels choose to maximize size while others opt for a time-minimizing

strategy (Vøllestad and Jonsson 1986; Vøllestad 1992; Poole and Reynolds 1996; Svedäng et al. 1996; Holmgren et al. 1997). Age at silvering is negatively correlated with growth rate and some variation is due to habitat differences (Svedäng et al. 1996). A correlation has been reported between length at metamorphosis and longitude as well as distance to the spawning ground (Vøllestad 1992). A clear trend in the proportion of migrating small and large eels throughout the migratory season was observed on the river Loire (Durif and Elie 2008). Those authors showed that the number of large eels increased regularly during migration, indicating that these large eels were located upstream in the watershed. Therefore, eels located further away from the spawning grounds were longer. Also of interest, is the fact that gonad development during hormonally induced sexual maturation increases exponentially with body length and eels over 70 cm show the highest gonad weight/body length ratio (Durif et al. 2006). Although female eels may silver at 50 cm (Fig. 2.1), they certainly benefit from attaining a greater size in terms of fecundity. The time needed to reach that size will vary according to their habitat and growth conditions. The extreme variability in length and age of female silver eels (Figs. 2.1 and 2.2) is
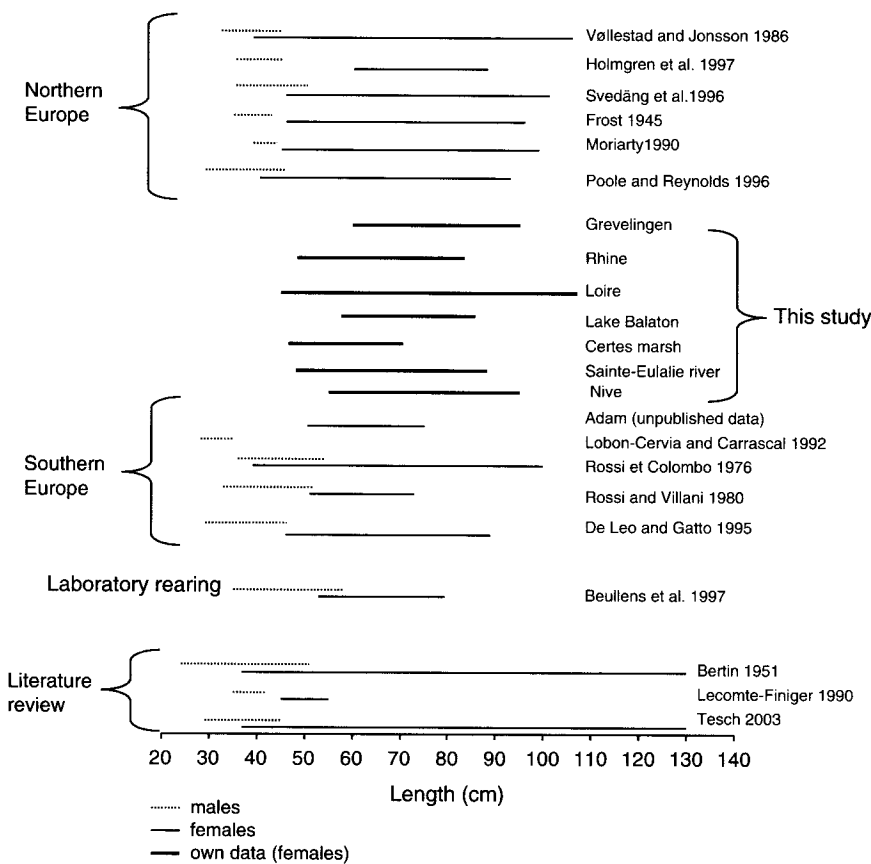

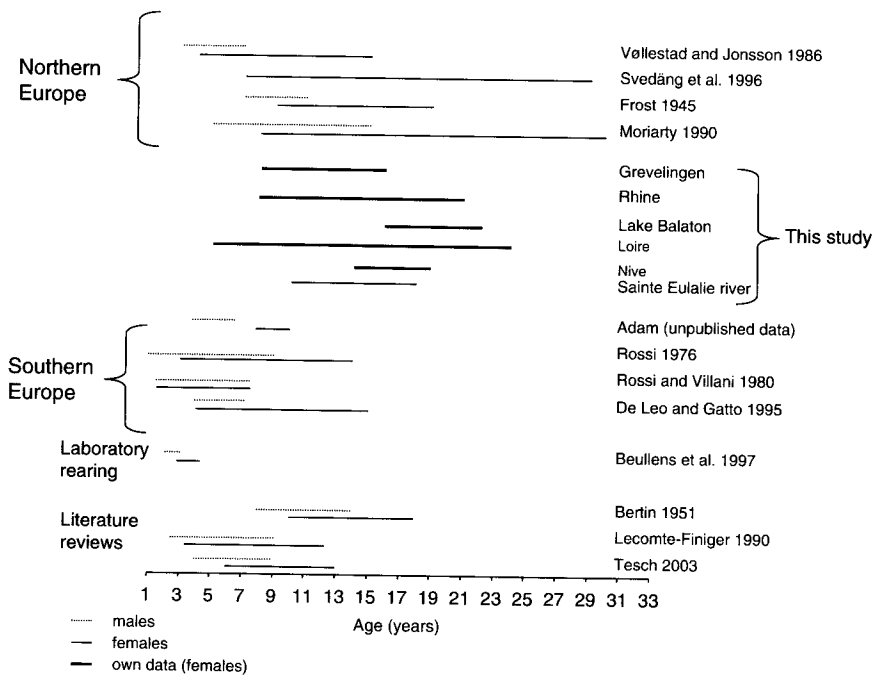**Fig. 2.1** Length of silver eels (*Anguilla anguilla*)

Fig. 2.2 Age of silver eels (*Anguilla anguilla*)

probably a reflection of the variability in habitats and growth conditions of eels. This variability is much reduced in males since they rarely grow over 50 cm (except when reared, Fig. 2.1). In the wild, they can start to silver at 30 cm, but most are around 35–40 cm. Their energetic demand obviously will not be as high as for females during reproduction.

2.3 Silvering Stages and Dynamics

As we have seen in the previous sections the overall characteristics of silver eels have been well documented. The modifications and differences between both yellow and silver stages are clear. Nevertheless, in the field one finds intermediate looking individuals that seem to fit both categories. Tesch (2003) writes that the best indication of whether the animal is physiologically a fully developed silver eel is the method by which it was caught. Thus little is known about the dynamics of silvering. Therefore, several studies were undertaken within the framework of the Eelrep project with the aim to give a fuller description of the silvering process by: (1) describing the events that lead to the migrating stage (2) gathering knowledge on the seasonal occurrence of these stages (onset and duration of silvering) and finally by (3) comparing different sub-population of silver eels from different locations.

2.3.1 Silvering Stages

Ecologists use clusters of individuals displaying similar characteristics to describe continuous phenomena. It then is easier to set boundaries to describe processes even when none are apparent. Thus the yellow and silver stages have been described as two separate stages whereas in fact the silvering process is gradual. Here we will give a description of the different stages eels go through during silvering. Further details of the study are given in Durif et al. (2005) and Durif et al. (in press).

Eels were collected at six locations in France which corresponded to different types of hydrosystems (rivers in both small and large catchments, small coastal river, estuary and marsh). Different types of fishing gear were used targeted on migrating individuals or not. Overall, 1,188 eels were sampled at different times of the year between 1996 and 2002. For each individual a profile was defined based on morphological and physiological characteristics. Table 2.1 shows the different measurements taken to define each profile based on previously described differences between yellow and silver eels.

Principal Component Analysis and Cluster Analysis were used to group individuals according to their profile (Fig. 2.3). Results of the analyses showed that many individuals displayed similar characteristics. The characteristics of the clusters that were defined by the analysis were consistent with a gradual transition from the yellow to the silver stage. Five different stages were determined for females that described the

Table 2.1 List of parameters measured and sampled on eels to define their morpho-physiological profile

| Process	Measurement	Index
Growth	Total body length	L
	Pituitary growth hormone	GH expressed as $\mu g\ g^{-1}$ of total body mass
	Age based on otoliths	Age in years
Fat stores	Body mass	Fulton's condition factor K
Increase in retinal surface area	Eye diameter	Pankhurst eye index EI
Increase of the pectoral fin	Pectoral fin length	Fin index: $I_F = $ fin length/L*100
Cessation of feeding: Regression of the alimentary tract	Mass of the digestive tract	Digestive tract index: DTI = mass of the alimentary tract/body mass*100
Development of gonads	Mass of gonads	Gonado-somatic index: GSI = mass of gonads/body mass*100
Development of liver	Mass of liver	Hepato-somatic index: HSI = mass of liver/body mass*100
Onset of puberty	Pituitary gonadotropin (GtH-II or LH-like)	LH expressed as ng g^{-1} of total body mass
	Vitellogenin	Log (VTG) expressed in $\mu g\ ml^{-1}$
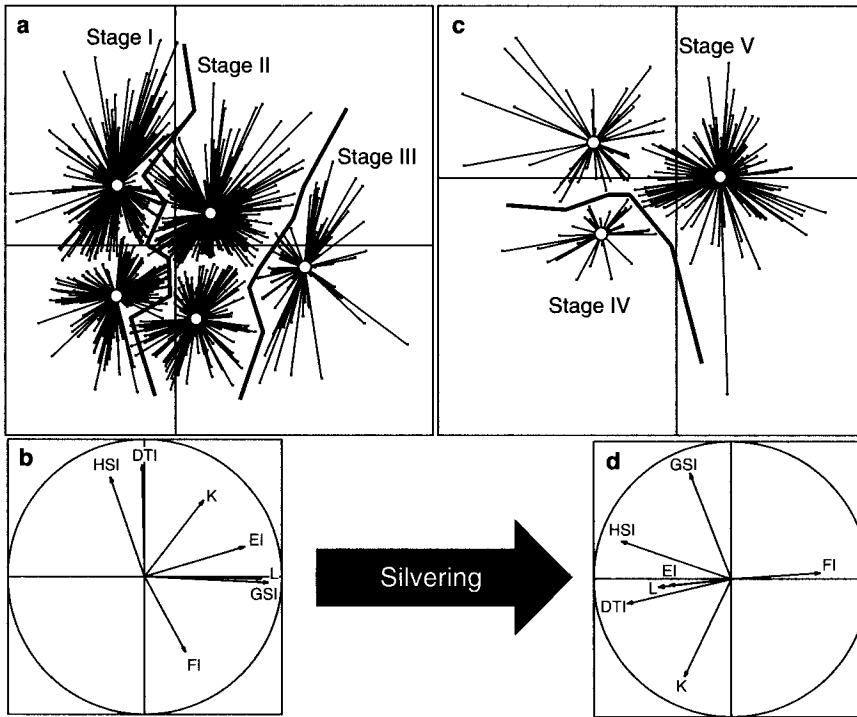
Fig. 2.3 Results of the principal component analysis and cluster analysis on female resident and migratory eels. Stars represent clusters of yellow (**a**) and (**c**) silver eels. (**b**) Correlation circle of the variables for yellow eels. (**d**) Correlation circle of the variables for silver eels. Broken lines in the factorial plots and separating the clusters correspond to the successive stages during the silvering process (Stages I to V). L: body length, K: Fulton's condition factor, EI: Pankhurst eye index, FI: fin index, GSI: gonado-somatic index, HSI: hepato-somatic index, DTI: digestive tract index

progression from small, undifferentiated yellow eels to pre-pubertal migratory eels. Figure 2.4 shows the evolution of the different morphological and physiological parameters for each stage in undifferentiated (stage I) and female (stages II to V) European eel.

Comparisons were made using ANCOVA and body length (for comparison of eye diameter and fin length) or body mass (for comparison of gonads, liver, and digestive tract masses) as a cofactor to remove any size effect. Bonferroni pairwise comparisons were used to detect which stages were different. Differences in vitellogenin, GtH-II (LH), and GH were detected using the Kruskal-Wallis test. Based on these results, the silvering process can be described as follows. Stages I and II correspond to the yellow stage, separating sexually undifferentiated individuals (stage I) from female eels (stage II). Eels at these stages have only slightly developed gonads, GSI is approximately 0.5% and under. There is not yet any production of vitellogenin or gonadotropin. Silvering is initiated at stage III, when vitellogenin shows a significant increase ($p < 0.05$). GtH-II (=LH)
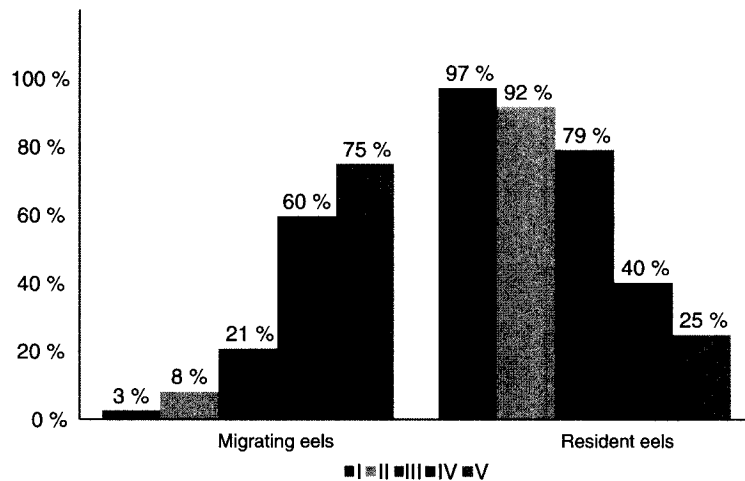
Fig. 2.4 Proportion of downstream migrating eels (caught in a downstream trap or a stow net) and resident eels (caught with fyke nets or eel pots) in each silvering stage

level shows a slight production. GH level reaches a maximum at this stage which suggests high growth rate before or at the onset of silvering. GSI of these pre-silver eels is approximately 0.8%. Eye diameter also increases significantly ($p < 0.05$) and mean eye index is 7.6. By backtracking the type of fishing gear used for each individual we found that most stage III eels in the sample (72%) had not yet begun their downstream movements since only 28% were caught with fishing gear targeting migratory eels (Fig. 2.4).

The silver migratory stage is reached at stages IV and V. Vitellogenin and GtH-II levels reach their maximum value as does the development of gonads (Fig. 2.5). The alimentary tract has significantly ($p < 0.05$) regressed and the eels have probably stopped feeding at this point. Eye index is around 10. Pectoral fin length continues to increase between stages IV and V. GH however decreases significantly from stage IV suggesting that at this stage, eels have switched from somatic growth to starting sexual maturation. The proportions of stage IV and V eels that were caught by specifically targeting migrants (captures at night with stow nets or trap during directed downstream movements) were respectively 68% and 82%.

2.3.2 Comparison of Silver Eels from Different Locations

Do migratory eels from different types of hydrosystems present the same morphological and physiological characteristics? Eight different locations were compared
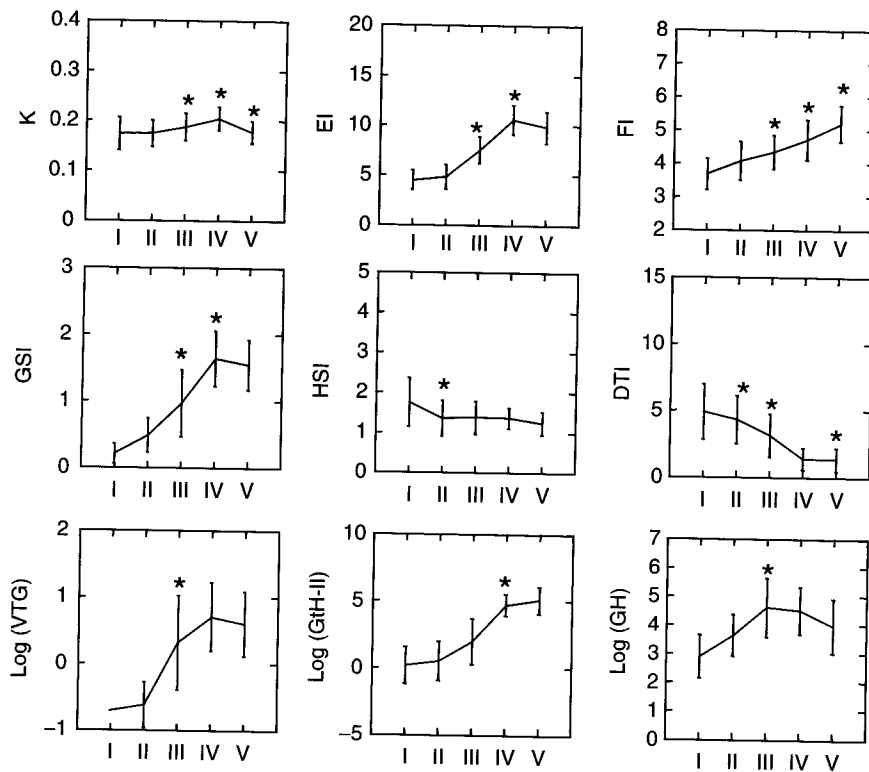
Fig. 2.5 Variations (mean ± SD) of certain morphological and physiological parameters during the silvering process, from resident eels (stage I) to female migratory eels (stage V). K: Fulton's condition factor, EI: Pankhurst eye index, FI: fin index, GSI: gonado-somatic index, HSI: hepato-somatic index, DTI: digestive tract index, vitellogenin, pituitary gonadotropin (gth-II), and GH: pituitary growth hormone. *: Significant difference from the value immediately before (P < 0.05), (see text for explanation of statistical tests)

(Table 2.2). Eels were sampled in the same manner as described above. Stages were assigned according to their physiological and morphological characteristics (Durif et al. 2008). Within the last silvering stage (V), we found small but significant differences in GSI according to the location (Fig. 2.6a). Landlocked eels of Lake Balaton in Hungary always displayed the lowest GSI, while eels caught in rivers during their downstream migration (Loire, Rhine and Sainte-Eulalie) showed the highest values. This suggests that high swimming activity (such as during downstream migration) further stimulates gonad development during silvering. Similarly, DTI values were lowest in actively migrating eels (Loire, Nive and Sainte-Eulalie).

Individuals obtained from the eel on-growing farm were all at stage III although they "looked" silver by their colour and eye size. Their mean GSI was 0.9% and

Table 2.2 Number of eels and origin

Location	Type of hydrosystem	Size of water-shed (km^2)	Abbreviation used in graphs	Fishing gear	Number of eels
Lake Balaton, Hungary	Freshwater lake (no outlet to the sea)	5,775	Ba	Fyke net, elec-trofishing	240
Certes, France	Brackish water marsh	155	Bw-Fr	Fyke nets, eel pots	108
Grevelingen, Netherlands	Brackish water lake	140	Bw-Nl	Fyke nets	86
Helmond, Netherlands	Eel farm	–	Farm	none	12
Loire River, France	river	110,000	Lo	Stow net	390
Nive, France	river	1,000	Ni	Downstream trap	61
Rhine, France	Large river	224,000	Rh	Fyke net	529
Sainte-Eulalie, France	Coastal fresh-water river	250	Ste	Downstream trap	61

none had undergone any regression of the digestive tract (DTI = 2.9%). During sampling it was found that most had food in their digestive tract. The only external feature that differentiated them from migratory eels at stage V was the length of the pectoral fin; fin index was 4 while it is approximately 5 in migratory eels (Fig. 2.6b).

2.3.3 Seasonal Changes

A detailed investigation of the seasonal dynamics of silvering was carried out on eels from the Grevelingen in the Netherlands (Van Ginneken et al. 2007a, b). The objective of this study was to obtain a finer seasonal description of the silvering process (start and duration) as well as a description of the physiological mechanisms involved (triggers and endocrine control).

Monthly samples of eels were collected between April and November in Lake Grevelingen. This is the largest brackish/saltwater lake of Western Europe with a total area of 14,000 ha. The lake is situated on the boundary between Zuid-Holland and Zeeland, the Netherlands and has a large standing stock of eels. Morphological, metabolic and endocrine parameters were measured.

Results showed a clear gradual increase in GSI and vitellogenin, but also in metabolites such as triglycerides, phospholipids, and cholesterol. Cortisol also showed a significant increase (one-way ANOVA p < 0.05) with highest values in September prior to downstream migration. From our observations in this study it became clear that a role for cortisol may be in mobilization of energy stores, especially in the European eel which has to cover a distance of 6,000 km to its spawning areas in the Sargasso Sea. Using large Blazka swim tunnels, (Van Ginneken and
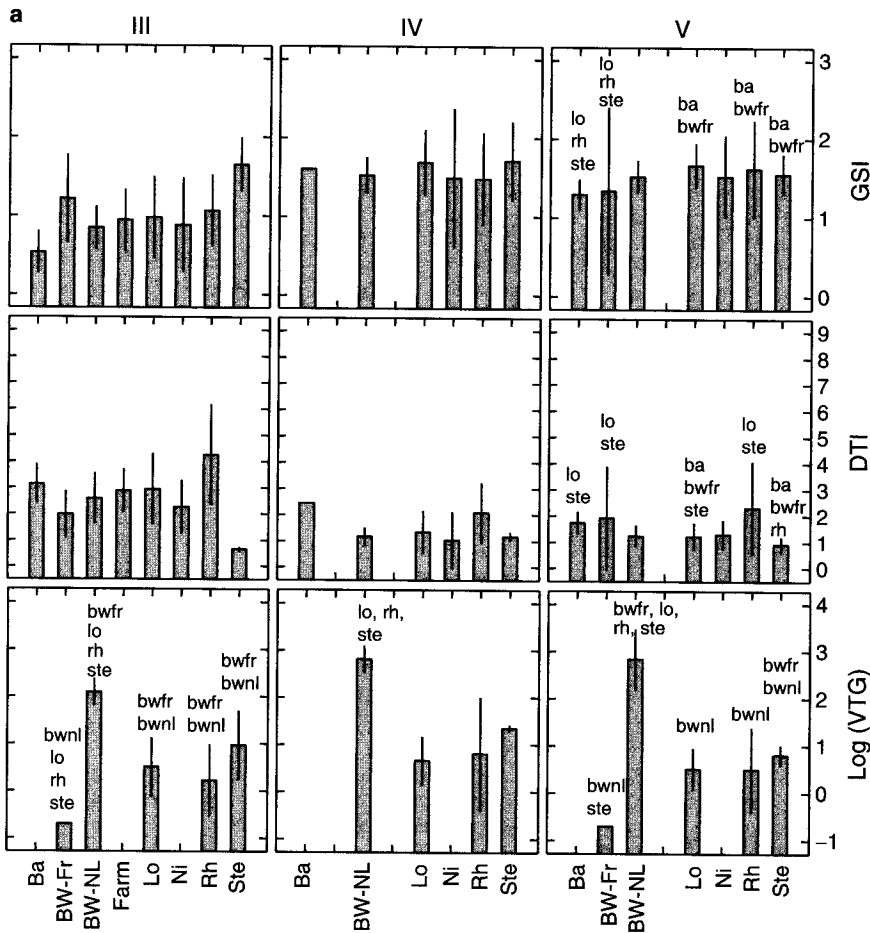
Fig. 2.6 a Variations (mean ± SD) in GSI, DTI and vitellogenin level in pre-migratory (stage III) and migratory eels (stages IV and V) from different locations. Explanations for abbreviations of locations are given in Table 2.2. Abbreviations on top of bars indicate significant differences (P < 0.05) with these locations

Van den Thillart 2000) demonstrated that for this tremendous swimming effort 40% of the energy reserves are needed while the remaining 60% can be used for gonad development. The observation that blood substrates like phospholipids and choles-terol are significantly (one-way ANOVA p < 0.05) increased in European silver eels corroborates the view that the major role of cortisol lays in the mobilization of the energy stores prior to and during migration.

August was clearly a cross-over month for silvering when stage IV and stage V eels appeared. A slight increase in T4 and T3 were found in eels caught in April. However it is not clear whether these changes were related to silvering, since such a cycle (minimum activity in summer and maximum activity in winter and spring) exists in many fish living at these latitudes (Swift 1960). Overall differences in T4
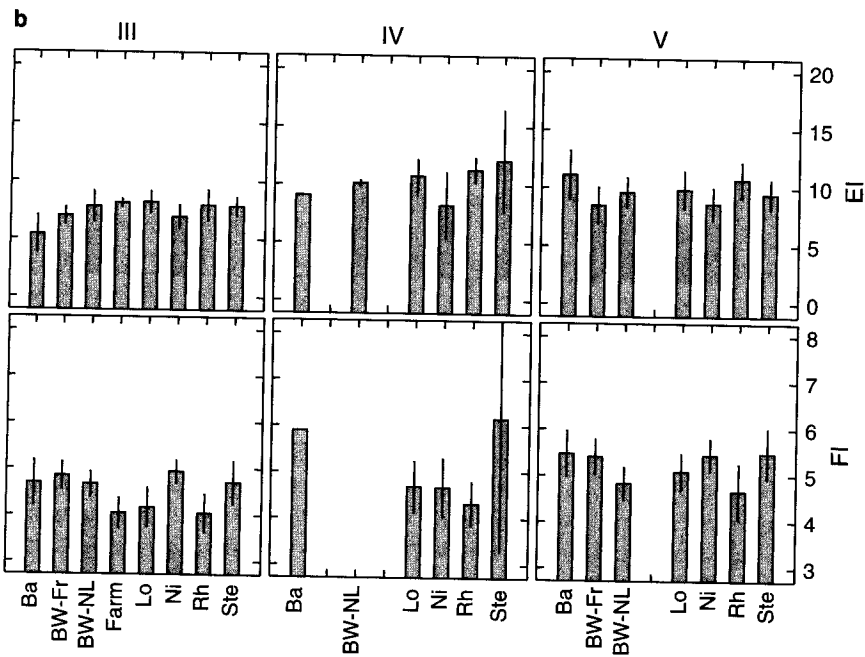
Fig. 2.6 (continued) **b** Variations (mean ± SD) in eye index (EI) and fin index (FI) in pre-migratory (stage III) and migratory eels (stages IV and V) from different locations. Explanations for abbreviations of locations are given in Table 2.2. No significant differences in these variables (P < 0.05) were detected between locations

and T3 were not significant between yellow and silver eels. A regular increase of 11-KT during silvering, with a maximum value in November during the migratory season was significant (one-way ANOVA, p < 0.05). It appears that androgens like testosterone play a more indirect role in reproduction in female fishes. They are produced in response to gonadotropin by the thecal layers of the oocytes and the levels gradually increase during oocyte growth and peaks during postvitellogenic growth (Nagahama 1994). We can conclude from the well-marked seasonal pattern in female testosterone, which lags behind but follows female estradiol (E2), that there is a relationship between the two (r^2 = 0.31). This apparent relationship between the two steroids supports the possibility that testosterone may act as a precursor for E2 synthesis during the vitellogenic season via aromatizing activity. The increased E2 profile in the period September-November suggests that in the period of gonad development the aromatizing enzymes are partially stimulated (Fig. 2.7).

In conclusion it appears clearly that silvering begins during the summer months (July–August). In the Grevelingen, changes coincided with a decrease in photoperiod and temperature. Analyses of commercial silver eel fishery data from the Loire River showed that the onset of downstream migration was linked to light level in terms of photoperiod and sunshine hours and migratory movements started earlier during years with low light level (Durif and Elie 2008).
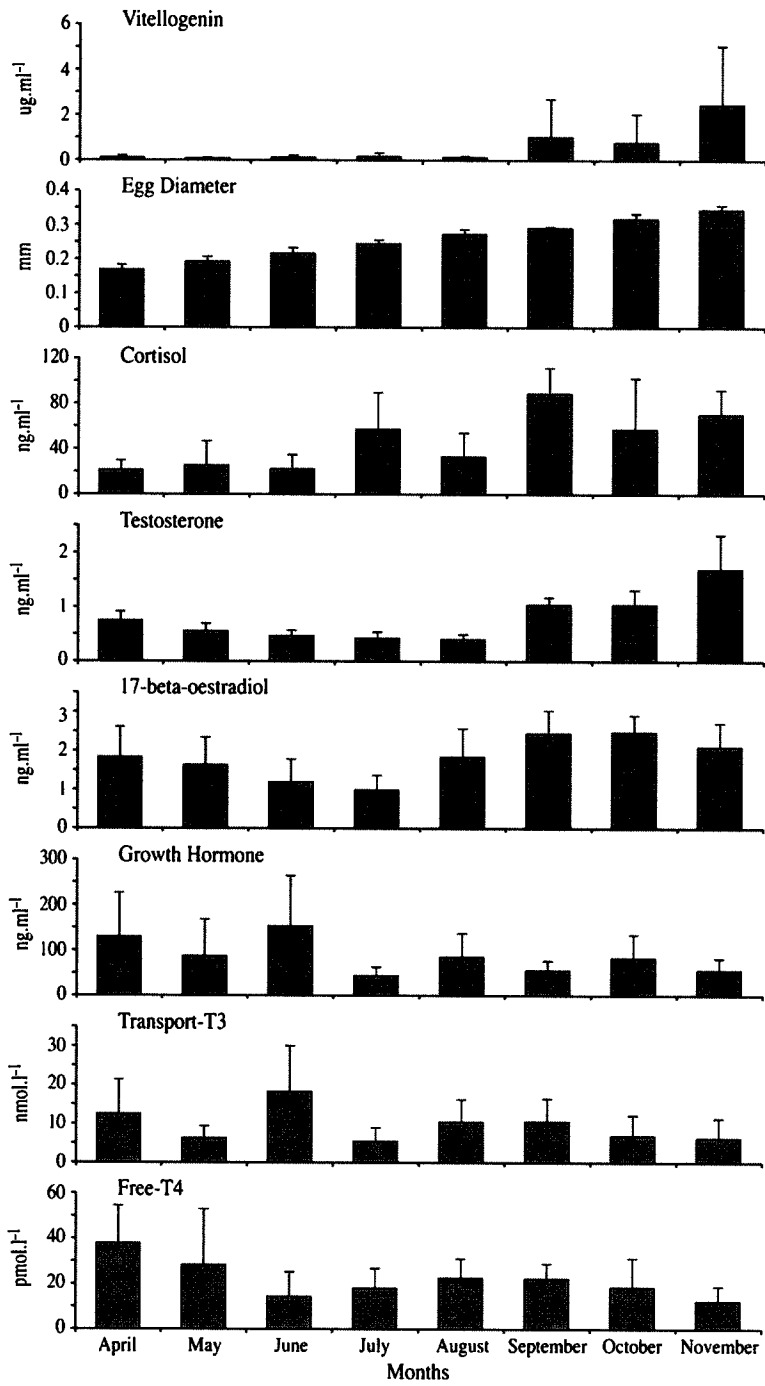
Fig. 2.7 Annual trends measured in eight female eels per month from Lake Grevelingen for maturation parameters and body hormones. April–July: yellow (sedentary stage) eels; August intermediate month; September–November: silver stage (migratory eels)

2.3.4 Age of Silver Eels

Age at silvering was obtained from most of the eels of the silvering stage study. The largest pair of otoliths (*sagittae*) was collected. Otoliths were later embedded in synthetic resin then polished to the nucleus with a polishing wheel. Etching was done using 10% EDTA. Yearly increments were revealed by staining with toluidine blue. Growth rings were then counted under a microscope. The age of individual eels was determined by the number of increments starting from the nucleus which was considered as year 1 of the eel's life.

Variability in age was extremely high. Resident eels (711 individuals) were on average 9.2 years old (min.: 3; max.: 21), pre-migrant eels (171 individuals) were 12 years old (min.: 6; max.: 22), and migrants (191 individuals) were 14.2 years old (min.: 5; max.: 24). The same variability existed within each location. Significant differences between locations were found within all stages (Fig. 2.8). Eels from the Loire were generally younger, especially resident eels with a mean age of 5.4, pre-migrant eels were 8.4, and migrant eels were 13.6. Landlocked eels of Lake Balaton were clearly on average the oldest eels obviously because they could not emigrate. Based on this, it could be hypothesised that eels would be older in large catchments where many obstacles were present. However this was not the case as migrating eels from smaller catchments such as the Nive and Ste Eulalie, were not younger on average then eels from the Rhine or the Loire (Fig. 2.8).

When body lengths of eels of the same age class were compared, migrant eels were always longer than resident eels indicating that migrating eels had benefited of a higher overall growth rate (Fig. 2.9). This suggests again that a high growth period precedes migration and therefore silvering.
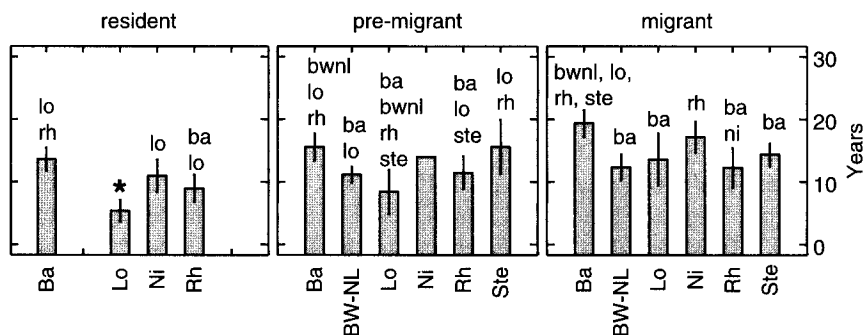

Fig. 2.8 Mean age (±SD) of resident (stages I and II), pre-migrant (stage III) and migrant eels (stages IV and V) from different locations: Lake Balaton (Ba), a brackish water lake in the Netherlands (BW-NL), Loire river (Lo), Nive river (Ni), Rhine river (Rh), Sainte-Eulalie river (Ste). Abbreviations (see Table 2.2 for explanation) on top of bars indicate significant differences (one-way ANOVA, $P < 0.05$) with these locations * indicates significant differences with all other locations (one-way ANOVA, $P < 0.05$)
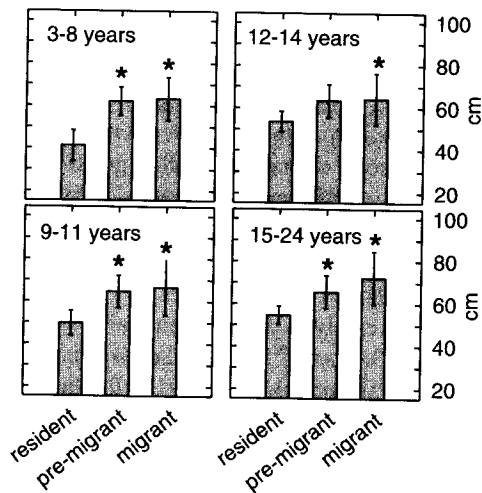
Fig. 2.9 Mean length (±SD) in mm of resident (stages I and II), pre-migrant (stage III) and migrant eels (stages IV and V) in different age groups. Age limits used to group eels were defined according to the 0.25, 0.50, and 0.75 quantiles of the age distribution of the sample. * significant difference with resident eels (one-way ANOVA, P < 0.05)

2.3.5 Changes in Skin Colour

Colour is the most obvious change during silvering. This criterion is most often used for stage determination (silver or yellow stage). Experienced fishermen or eel researchers can tell by its external features whether an eel is migratory or not. However, it is difficult for one to say which criteria are actually used in this rapid designation. Colour visualisation can be different from one person to another. The change in skin colour is more or less obvious. Countershading is not always present and tones are sometimes intermediate between yellow and a bronze color. We investigated to what degree these changes in colour were related to "maturity" of eels. A spectrophotometer (Minolta CM-508d) and CIELAB system were adjusted for reflectance, illuminant D65, and angle of 10°. Three measurements were taken on 194 individuals (comprising stages I, III, IV and V eels): on the back, on the belly, and just below the lateral line. Luminance (L^*) represents the degree of blackness (black = 0; white = 100). Red is represented by + a^*, green by $-a^*$, yellow by + b^*, and blue by $-b^*$.

Colour measurements on the back of eels could be directly linked to silvering. We found significant correlations (Pearson correlations, P < 0.05) between colour measurements on the back and GSI (gonado-somatic index), DTI (digestive tract index), and EI (eye index). L_{back}^* was significantly correlated (p < 0.05) with GSI ($r^2 = 15$), DTI ($r^2 = 0.27$), and EI ($r^2 = 0.20$). Therefore, eels with a dark back (low L^*) had a high GSI, a low DTI, and a high EI. Significant correlations were also found with b values (yellow) on the back. Yellow decreased with GSI ($r^2 = 0.20$), and eye index

($r^2 = 0.24$), and increased with DTI ($r^2 = 0.36$). Values of $a*$ also showed significant correlations with DTI ($r^2 = 0.20$) and EI ($r^2 = 0.20$) indicating that regression of the digestive tract and eye size increased as "red" or bronze appeared on the back.

For each individual we evaluated the colour difference (ΔE) with a "yellow eel" using the Hunter-Scofield equation, which represents the distance in the *Lab* space between two colours: $\Delta E = ((\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2)^{1/2}$. Control values for the "yellow eel" were obtained from averaging the $L*a*b*$ values of stage I eels. Thus we obtained three values for each eel: $\Delta E_{back}, \Delta E_{lateral\ line}$ and ΔE_{belly}. Results showed that there was high individual variability within each stage. Only ΔE_{back} showed significant differences between stages (Kruskal-Wallis test, $p < 0.05$). The colour change was perceptible starting at stage IV since there was no significant difference between stage I and stage III eels. Stage IV showed the highest colour difference with "yellow eels" (Fig. 2.10).

2.3.6 Regression to a Yellow Stage

It is not known whether eels that are prevented from migrating go back to a "yellow" stage. Here, we investigated whether silver eels held captive beyond their normal migration, recovered "yellow" eel characteristics.

The first experimentation involved 104 female eels captured in November 2000 by a professional fisherman on the Loire River in France. Gonads were initially sampled on 29 individuals at the site of the commercial silver eel fishery. Mean GSI
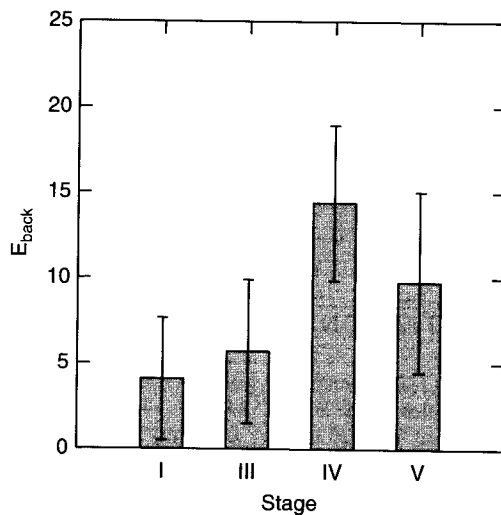

Fig. 2.10 Variations (mean ± SD) in colour differences measured on the back (δe_{back}) of different staged eels (resident: stage I, premigratory: stage III, and migratory stages IV and V)

of freshly caught migrating silver eels was equal to 1.8. The remaining 75 eels were placed in seawater tanks. After 2 months in the tank (January), GSI of eels showed a slight but significant decrease (Fig. 2.11). Colour values were still equivalent to measurements done at the fishery. In May (more than 6 months later), mean GSI was significantly lower (Mann and Whitney test p < 0.05), and it reached values corresponding to those found in yellow eels (lower than 1%). Skin colour also changed throughout the experiment. Values measured on the back of eels showed important variations at the very beginning of the experiment which were probably linked to handling of eels and to their change of environment: Luminance (L*)
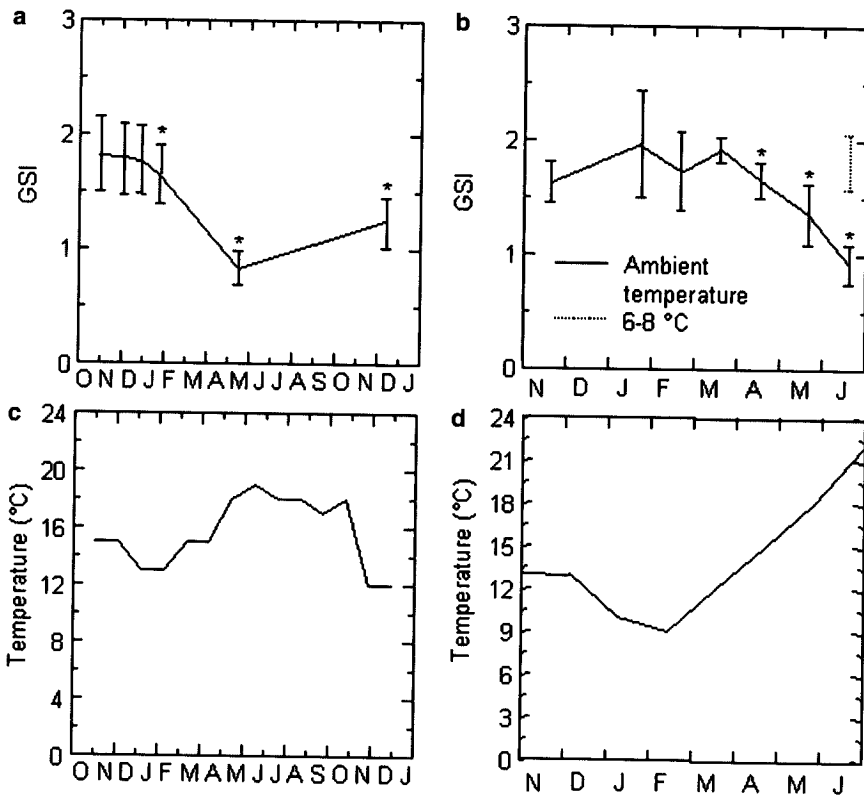

Fig. 2.11 Variations in GSI (±SD) of migratory eels which remained captive beyond their season of migration. Months are labeled on the x-axis. * indicates a significant difference from the value immediately before (Mann and Whitney test P < 0.05). (a) First set of experiments (November 2000–December 2001). Initial sampling was performed at the fishery (n = 29). Then eels were sacrificed at regular intervals (n = 46; n = 7; n = 7; n = 3; n = 13). (b) Second experimentation (November 2002–June 2003). Initial sampling was performed at the fishery (n = 22). Following samples respectively correspond to n = 5; n = 4; n = 5; n = 6; n = 6; n = 5 (ambient temperature). Eels kept at 6–8°C represent three individuals. (c) Mean monthly water temperature (2000–2001); (d) mean monthly water temperature (2002–2003)