CULTURE OF SOLE, *Solea senegalensis* KAUP. HISTOMORPHOLOGICAL AND HISTOPATHOLOGICAL ASPECTS

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1.–THE CULTURE OF *SOLEA SENEGALENSIS*

Solea senegalensis is a common sole of Mediterranean waters, and it represents 95% of the sole catch in the southern part of Portugal. It is very similar to *Solea vulgaris*, a flatfish common in Atlantic waters. Morphologically, these two species are extremely close and larvae are very difficult to distinguish (Lagardère 1979).

Both species are gonochoric and the first maturation for the females is achieved at age 2^+ for *S. senegalensis* and 3^+ for *S. vulgaris*, when the total lengths are 32 cm and 30 cm respectively (Dinis, 1986). Ramos (1982) reported 27 cm as the total length at first maturation for *Solea vulgaris* in the Mediterranean area. The spawning season is late winter for *S. vulgaris* (January-March) and spring for *S. senegalensis* (April-June) (Ramos, 1982; Dinis 1986; Andrade 1990). Fecundity for both species is very similar, with 509 oocytes /g fish for *S. senegalensis* and 530 oocytes/ g for *S. vulgaris*. Both species have a high market price, and they are now alternative species in marine aquaculture.

Solea senegalensis is well adapted to warm climates. It is commonly exploited in the extensive coastal aquaculture utilising earthen ponds along the south coasts of Portugal and Spain (Drake *et al.*, 1984; Rodriguez, 1984, Dinis 1986, 1992). The life cycle is similar to that of the better known *Solea vulgaris* (Russel, 1976) and the growth rate of *Solea senegalensis* under extensive polyculture conditions is higher than that of *Dicentrarchus labrax*. Only *Sparus aurata* presents a better growth (Drake *et al*, 1984)

The reproduction in captivity of *Solea senegalensis* has been the subject of research in Spain and Portugal since the early eighties (Rodriguez and Pascual, 1982, Rodriguez 1984, Dinis 1986,1992). Due to bad results concerning weaning and growth of juveniles marketable size (Metailler *at al*, 1983, Dinis, 1992) there was a decrease in the perceived importance and potential for aquaculture, subsequently resulting in a decrease in the research studies.

However, due to a decrease in the profitability of sea bream and seabass cultivation resulting from over - production in the Mediterranean area research efforts have been renewed.

As a consequence, a big effort among Portuguese and Spanish research groups has been made during the last three years, in order to solve the principal problems of the cultivation of *S. senegalensis*, such as the nutritional problems related with the weaning, and pathology related to pigmentation of juveniles.

The cultivation procedure as well as the pathological alterations during the production of juveniles will be presented.

1.1.–Broodstock Management

The reproduction of marine species in captivity depends on numerous environmental factors, and the induction of spawning may be hormonal or by manual stripping. In Soleidae experiments with HCG in *S. vulgaris* (Girin 1979), hypophysis extracts of carp and tuna (Rodriguez and Pascual, 1982) and LH-rH (Dinis, 1986) in *Solea senegalensis*, did not give positive results, either because the fish did not show any gamete emission, or because the quality of eggs was very poor. Although good results have been obtained with stripping for other flatfish such as turbot, the technique is not feasible in *Solea spp.*

Therefore, natural spawning is the only way to obtain viable eggs. The quality of the broodstock,

methods of capture and conditioning methods (environmental factors and nutrition) have been found to be important in obtaining viable gametes.

1.1.1.–Capture

The adults of *S. senegalensis* are very sensitive to methods of fishing, because nets easily damage them. Devauchelle (1980) recommended "la capechade", but we have obtained good results with net traps during the night. Trammel nets should be avoided, but short trawls or beach seines may be used.

1.1.2. –Adaptation to captivity

During the first week the fish should be kept in tanks with a thin bottom layer of sand and in the dark, in order to improve adaptation, since this species is active nocturnally.

The use of already mature broodstock is not possible, because either the mature females have a low survival or they reabsorb the gonads. It is therefore advisable to set up the stock during the interspawning period, which is between July and December.

The identification of sexes is difficult during this period because there is little external differentiation, but with some experience it is possible to identify sexes. Stock density should be 1 - 1.5kg/ m^2 surface.

1.1.3.–Feeding regime

Studies on stomach contents of *Solea senegalensis*, showed a dominance of Polychaetes (*Hediste diversicolor*) and Amphipods, Copepods and Isopods were also identified (Bernardo, 1990, Arias and Moyano, 1990, Drake and Arias, 1989). The results of Dinis (1986) based on the work of Fluchter & Trommsdorf (1974), showed the importance of Polychaetes in the feeding regime in captivity of *S. senegalensis*. The feeding regime is therefore, based on Molluscs (*Loligo vulgaris*) and Polychaetes (*Hediste diversicolor).*

1.1.4.–Pathology

The ectoparasite *Hemibdella solea* (Hirudinae) (Burneson *pers. comm.*) has been identified. This parasite seems not to affect the fish. Although the density of the parasite may reach around 20/cm^2 on spawners, no harmful effects were noticed on the fish. This parasitic leech also infests *Solea vulgaris* (Baynes *et al*, 1993). The control of this parasite is through salinity. Low salinity detaches the parasites, preventing the completion of the life cycle.

Sporadic outbreaks of pasteurellosis (*Pasteurella sp.*) have been detected in a group of wild broodstock introduced into a fish farm and submitted to a hormonal manipulation (Sarasquete *et al*, 1993)

1.2.–EGGS AND LARVAE

1.2.1.–Incubation

Pelagic eggs were collected using surface collectors and incubated in the same circuit as the broodstock in order to maintain the same temperature and salinity as during spawning (Fig.1).

After hatching, the larvae a density of 100 larvae/litre, were transferred to cylindroconical 200 litre fibreglass tanks, in a closed system, where salinity was maintained at 35% and temperature ranged from 16.5 ± 0.5ºC to 22± 1.0ºC.

1.2.2.–Larval development

The larvae accept *Artemia* nauplii at first feeding, but the results of Magalhães and Dinis (1996) showed that there are no significative differences in growth between a feeding regime based only, on Artemia or with Rotifers and Artemia. However most research continues to use the regime in which larvae are fed on day 3 using Rotifers (*Brachionus plicatilis*) as first prey, followed by *Artemia* nauplii (Dinis, 1992).

The larvae hatch at an average size of 2.42 ± 0.09mm total length (Fig 2), and at first feeding

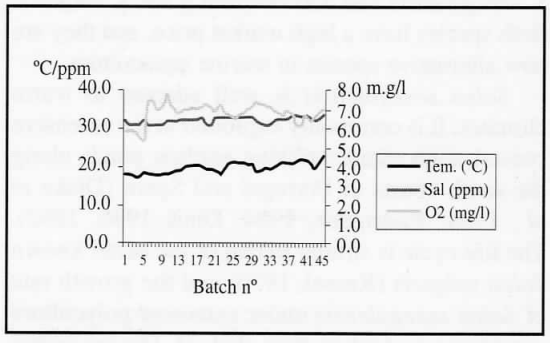

FIGURE 1. Environmental spawning parameters

3.34 ± 0.08mm. They were fed on *Brachionus plicatilis* (5br/ml) enriched on 50% *Isochrysis galbana* and *Chlorella spp.* from day 3 to day 5 DAH, and newly hatched *Artemia* nauplii from day 4 to day 15. Enriched *Artemia* metanauplii were given "ad libitum" from day 12 to day 30.

The metamorphosis started on 11DAH and was completed on 19DAH, at which the larvae were transferred to flat bottomed 200 litre fibreglass tanks. Poslarvae remained in these tanks until 40DAH when they were transferred to outdoor earth ponds for ongrowing or for weaning experiments with inert foods.

The growth curves for total length and total weight for the larval period are shown in Fig.3.

Survival at day 19 ranged from 28.6% to 87% when the metamorphosis had finished and the fish were moved to flat bottom tanks.

1.3.–Weaning period

From day 19 until day 40, fish were fed on *Artemia* metanauplii while deep frozen *Artemia* was given "ad libitum", providing inert food for the fish already with a benthic activity.

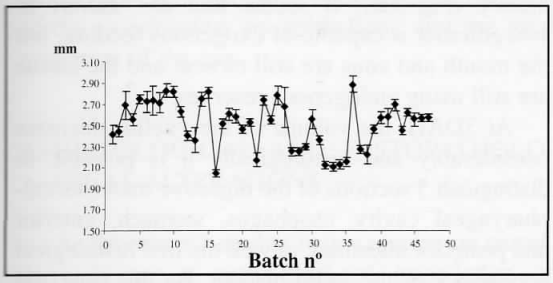

Figure 2. Newly hatched larvae - Total length

Reports the starting of the weaning period differ among the authors.

Appelbaun (1985) reported that *Artemia* has a dual role: as food and in the provision of digestive enzymes. The same author emphasised that the longer the period with *Artemia* the better the growth and survival in sole, but that this regime makes the fish difficult to wean into inert food.

With *Solea vulgaris* Gatesoupe & Luquet (1982) started weaning at 10DAH, Fuchs (1982) at 25DAH and Person-Le Ruyet (1980) 30-40 DAH. With *Solea senegalensis*, Dinis (1992) started with larvae 30 DAH using rehydratable pellets. Flos *et al.* (1995) started with juveniles of 6 months. Despite poor results, the authors agreed that early weaning should be balanced between costs and survival. Flos *et al.* (1995) propose the inclusion of enzymes in the diets in order to be able to start earlier, by providing the means by which the food can be digested.

During 1996, experiments of weaning 40DAH juveniles of *Solea senegalensis* were carried out, using laboratory moist pellets composed of commercial pellets incorporating *Artemia* and gelatine.

The survival was very poor, the fish died after two weeks, despite of an initial good acceptability of the food.

The growth in weight and length is shown in Fig 4

The specific growth rate (SPG) and the condition index during the weaning period are

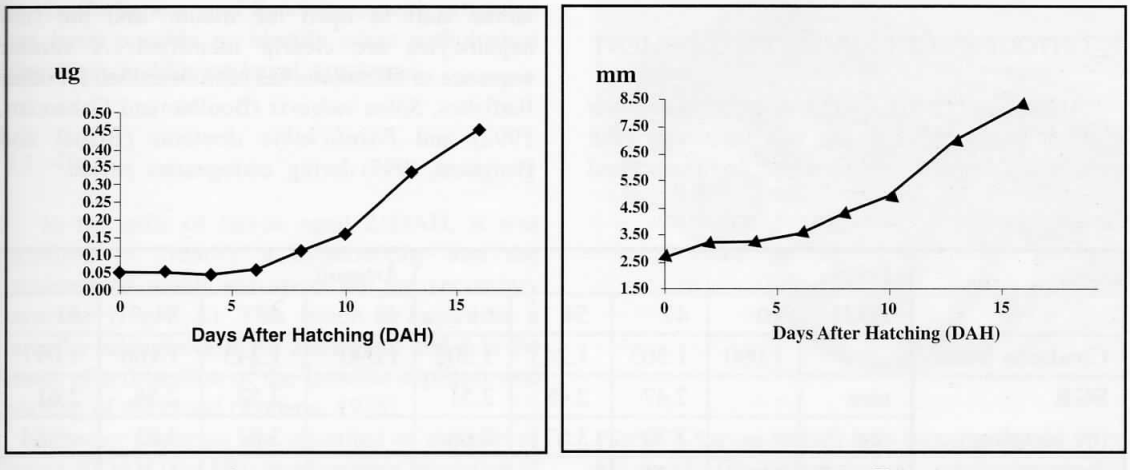

Figure 3. *Solea senegalensis* larvae - Growth in weight (**A**), growth in length (**B**)

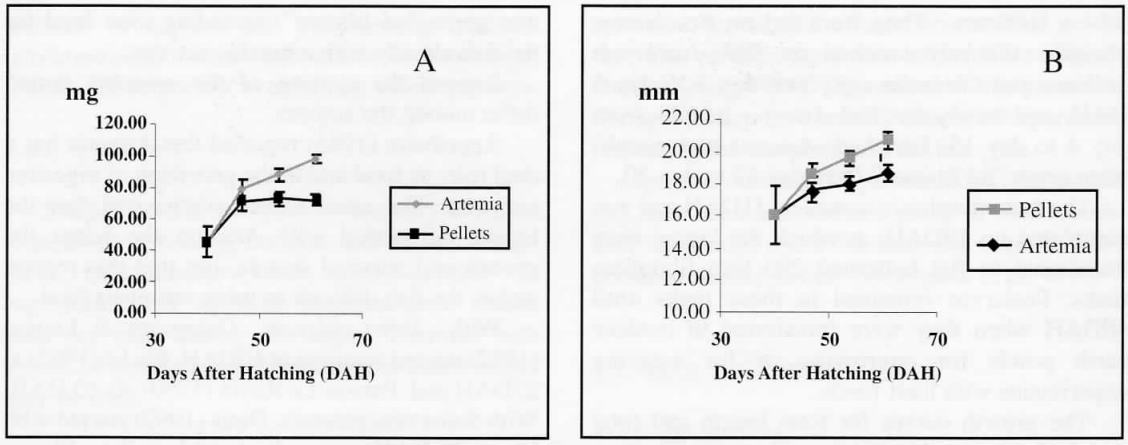

FIGURE 4. Weaning of *Solea senegalensis* - Growth in weight (**A**), growth in length (**B**)

shown in Table 1, and didn't differ significatively between the treatments (F> 3.09)

1.4.–PRINCIPAL BOTTLENECKS

The weaning period for this species produces high mortalities. The benthic fish is not very active and may resist long periods of starvation (Fuchs, 1982). The use of rehydratable pellets treated with attractant substances was tested (Dinis *et al*, 1987, Metailler *et al*, 1983), but survival and growth was very poor, around 33%. More recently the incorporation of hydrolysed fish protein concentrate into weaning diets for *Solea vulgaris* (Day *et al* 1997) has resulted in survival of between 75.5% and 42.5%.

2.–ONTOGENY OF LARVAL DEVELOPMENT

At hatching (1DAH) the digestive tract is a tubular segment laying dorsal to the yolk sac, with both extremities closed, and with undiferenciated structures This epithelium consists of a monostratified layer of cubic/columnar cells. The yolk sac contains several peripheral oil globules (Fig 5A).

At 2DAH it is already possible identify the first primordial structures, such as the oesophagus, and the primordial cells of the liver, pancreas and kidney (Fig 5B). It seems that at 2DAH *S. senegalensis* is capable of exogenous feeding, but the mouth and anus are still closed, and the larvae are still using endogenous reserves.

At 3DAH, the volume of the vitellus decrease considerably and histologically it is possible to distinguish 5 sections of the digestive tract (buccal-pharyngeal cavity, esophagus, stomach, anterior and posterior intestine), as well the first histological appearance of the swim bladder. By this time, the loop of the digestive tract is formed, occupying the right side of the larvae (Ribeiro et al, 1997). Some larvae start to open the mouth, and the first hepatocytes are clearly identified. A similar sequence of alterations has been described for other flatfishes, *Solea vulgaris* (Boulhic and Gabaudan, 1992) and *Paralichthys dentatus* (Bisbal and Bengtson, 1995) during endogenous period.

TABLE 1

	Pellets					*Artemia*			
	DAH	40	47	54	61	40	47	54	61
Condition Index		1.090	1.303	1.243	1.202	1.090	1.243	1.166	1.097
SGR	mm	-	2.47	2.48	2.51	-	2.52	2.56	2.61
(by sampling)	mg	-	3.72	3.67	3.66	-	3.82	3.85	<3.84
SGR	mm	2.79				2.90			
(40-61 DAE)	mg	4.09				4.40			

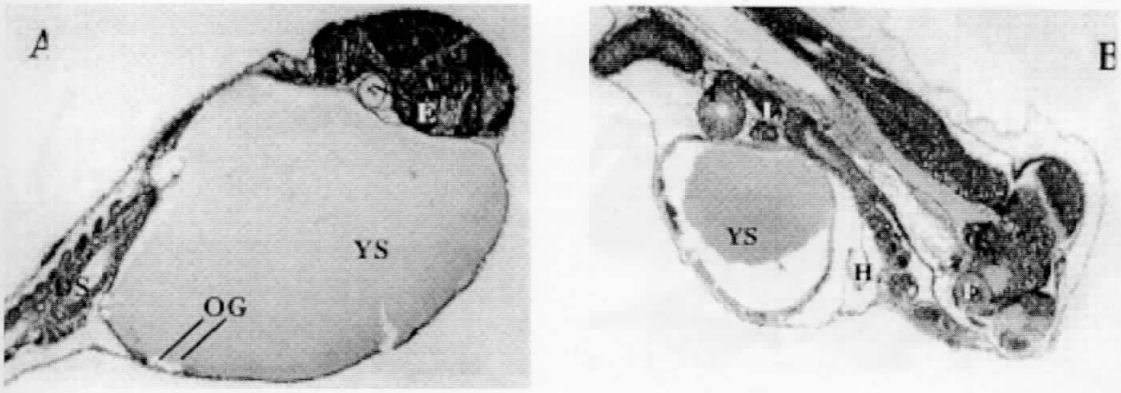

FIGURE 5. Newly hatched larvae (**A**) and Larvae 2DAH (**B**) YS - yolk sac; OG - oil globule; DS - digestive system; E - Eye; L - liver; I - intestine; H - heart

At 12DAE (Fig. 6) there is differentiation of the eye, which shows different layers. At this stage, the swimbladder is already inflated, with the gas gland formed, and the gills possessing the primary and the secondary lamellae

Metamorphosis is achieved at 19DAH (Fig 7) but at 18DAH it is possible to observe the invasion of the swimbladder by epithelium, and the total migration of the eye.

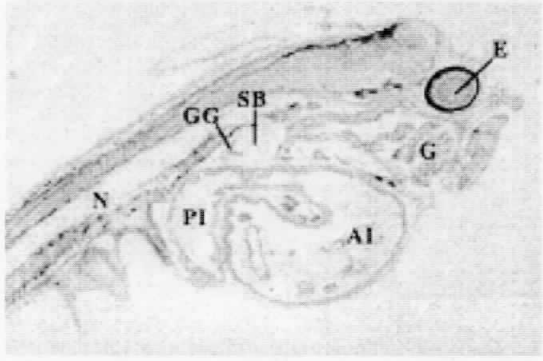

FIGURE 6. Larvae 12DAH E - eye; G - gills; SB - Swim blader; GG - gas gland; AI - anterior intestine; N - notochord;

3.–HISTOLOGICAL AND HISTOPATHOLOGICAL ALTERATIONS

As the research studies in this species are recent, there is not a great deal of knowledge of pathological alterations. However, during the research studies carried out during the last two years by the Portuguese and Spanish research groups, it has been possible to identify some pathological alterations in different larval structures.

3.1.–GILLS

In the gills of larvae aged 21DAH, it was possible to identify a hypertrophy and the presence of small red spots on the secondary lamellae (Fig 8 A). This lesion is known as a lamellar telangiectasis (or aneurysm) and it is the result of a dilatation of the lamellar capillary and pooling of the blood (Roberts, 1978).

Another alteration was identified on the gills of larvae 40DAH (Fig 8B): an edematous separation of the respiratory epithelium with a necrosis of the epithelial cells at the base of the secondary lamellae.

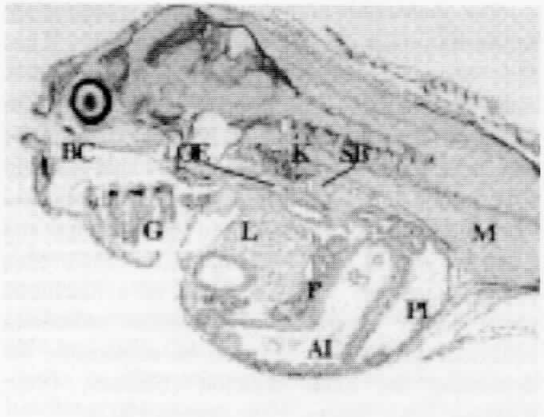

FIGURE 7. Larvae 19DAH BC - buccal cavity; L - liver; M - muscle; OE - oesophagus; AI - anterior intestine; PI - posterior intestine; P - pancreas; K - Kidney; SB - swim bladder, G - gills

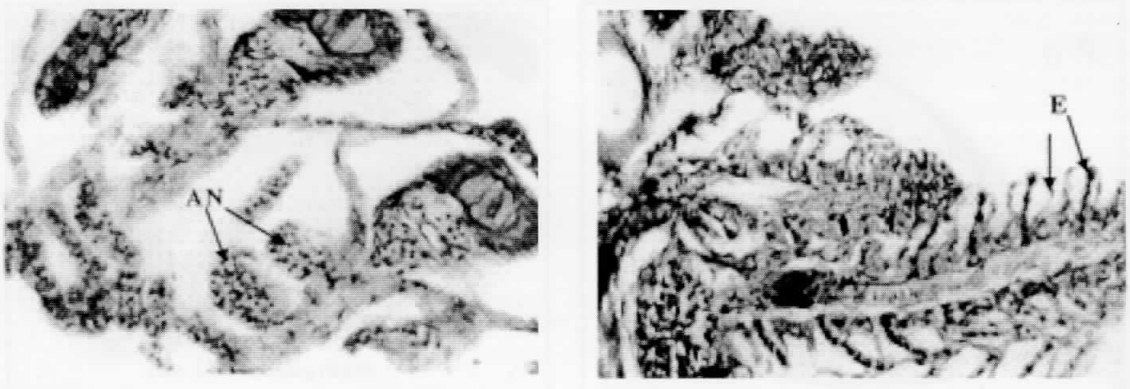

FIGURE 8. Gills *Solea senegalensis* of 21DAH (A) and 40DAH (B) AN - aneurysm; E - respiratory epithelium

These histopathological alterations are usually related with high values of metabolic wastes in the water (Spotte, 1992), and are commonly found in farmed fish. The fish gills are easily alterated by external irritants, such as heavy metals, pesticides and ammonia. The oedematous separation of the respiratory epithelium is due to in increase of capillary permeability (Roberts, 1978).

3.2.–SKELETON

In Mediterranean aquaculture the standardisation of controlled reproduction and larval rearing systems has led to an increased in production of fish larvae. The progeny show, however, especially in seabass and sea bream some morphofunctional anomalies, which cannot be entirely eliminated by larval selection.

The skeleton malformations have an important consumer impact, causing a lower market price. It has been suggested that the cause of such deformities could be the poor rearing conditions, related to nutritional deficiencies such as Vitamin C. Lacks of this vitamin can affect the collagen metabolism in bones, provoking serious lordosis and scoliosis, and this relation has been identified in carp (Mahajan and Agrawal, 1980) and in seabass (*Dicentrarchus labrax*) (Godeluc, 1983). Absence of a functional swim bladder which provokes aberrant swimming behaviour in the fish can also be responsible for deforming the axial skeleton (Chatain, 1989; Kitajima, 1981; Soares, 1996; Soares, 1997).

The increased interest in the mass production of juveniles sole, has led to the identification possible skeleton alterations similar to those previously described.

The larvae were cultivated using the normal standard procedures for the species. Samples were collected and fixed in formaldehyde (4%). Cartilage and bone were stained with Alcian Blue and Alirazin Red S, according to Dingerkuss and Uhler (1977) and Hanken and Wasserry (1981). Alcian Blue is specific for acid mucopolissacarids and Alirazin Red for calcium.

At hatching the notochord is the only structure responsible for larval support.

The first cartilaginous structures were observed at 18DAH when exogenous feeding started. At this time Meckel's cartilage and the optical capsules are clearly stained (Gavaia, 1997).

The first ossified structures, were observed. The cleithum is stained pale pink, indicating start of calcification, and the first cartilagineous supporting arcs of the caudal fin (hypurals) are also observed. (Fig 9 A). At this age the larvae initiate benthic morphology.

Later, at 24DAH (Fig 9B) the beginning of calcification of the dorsal column is well marked, (the pale red of the dorsal column). This ossification takes place in the antero-posterior direction.

The ossification is completed at 39DAH when the fish is exclusively benthic. At this stage it is possible to identify some skeleton alterations, the reasons for which are, as yet, unknown.

Among the most characteristic alterations those related with the fins arcs were the most common (Fig 10)

3.3.–SWIM BLADDER

Abnormal swim bladders in physoclist species have been related to lordotic deformation,

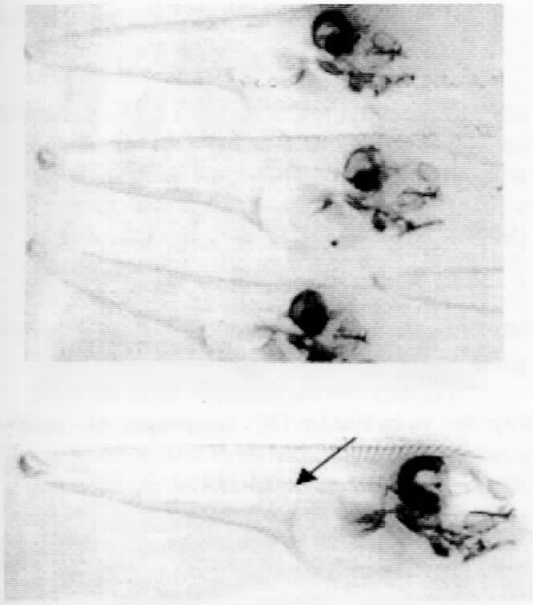

FIGURE 9. Cartilaginous skeleton larvae 18DAH (A); Beginning of calcification at larvae 24DAH (B)

decrease of growth rate and larval mortality (Dinis *et al*, 1997)

Since flatfishes have a functional swim bladder only during pelagic life, the non-functional swim bladder is normal found in juveniles.

At 3DAH it is possible to observe the first histological appearance of the swimbladder. (Fig 11 A and B)

The swim bladder is inflated (=functional) in pelagic larvae aged 11DAH (Fig 6)

At the migration of the eye started at 13DAH, it was possible to identify the existence of a group of epithelial cells in the lumen of the bladder. On a more advanced development stage, the lumen of the swim bladder is filled with epithelial cells, and the larvae in this stage is or are benthic. (Fig 12).

3.4.–SKIN

As in other species the skin of the newly hatched sole larvae, , consists of only two layers of squamous epithelial cells through which gas exchange probably takes place, since the gill filaments are not yet developed (Fig 13A). A large fluid-filled space separes the epidermis from mesoderm (Fig 13B).

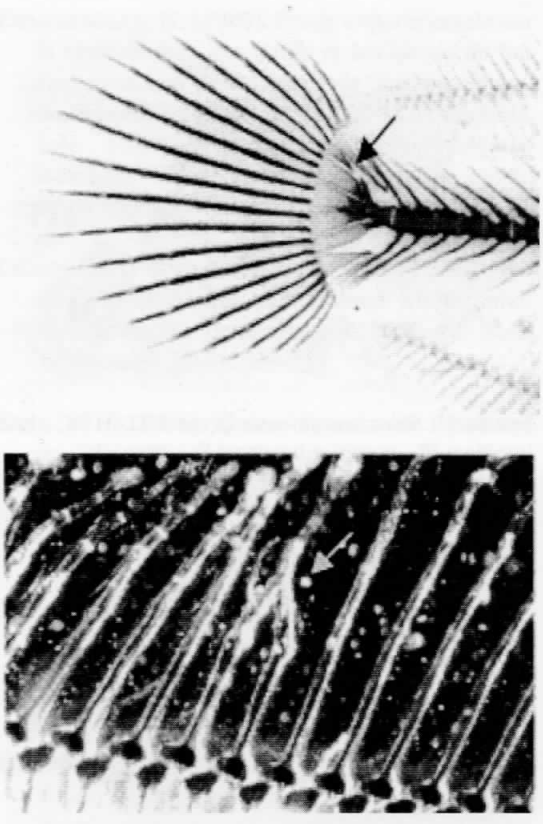

FIGURE 10. Skeleton alteration in the caudal fin **A** (juvenile 45DAH) and dorsal fin **B** (juvenile 75 DAH)

At the end of metamorphosis, about 17-20 DAH there is a big change in the ecology and physiology of the fish due to the change to benthic habits, and it might be expected that changes would occur in the developing skin.

During larval development, epidermal sacciform, as well as branchial and epidermal chloride cells, were unreactive to cytochemical tests of carbohydrates and proteins. Padros (1994) suggested that in *Scophthalmus maximus* the sacciform cells are possibly transformed during development, into active mucous secreting cells. After metamorphosis, *Solea senegalensis* presents numerous mucous or goblet cells containing strongly sulphated glycoproteins in the corporal skin and gills, (Sarasquete *et al*, 1998).

As in other teleosts senegalese sole larvae at hatching, (Morrison, 1993; Padros 1994), have numerous neuromasts on the epidermal head epithelium (Fig 14).

In *Scophthalmus maximus* larvae (Padros, 1994) the presence of neuromasts have been

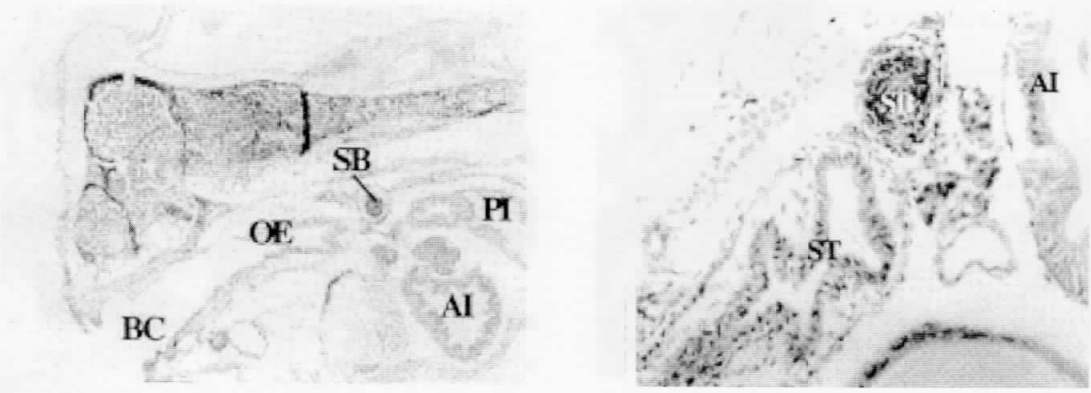

FIGURE 11. *Solea senegalensis* larvae 13DAH BC - buccal cavity; Sb - swim bladder; OE - oesophagus; AI - anterior intestine; PI - posterior intestine; St - stomach

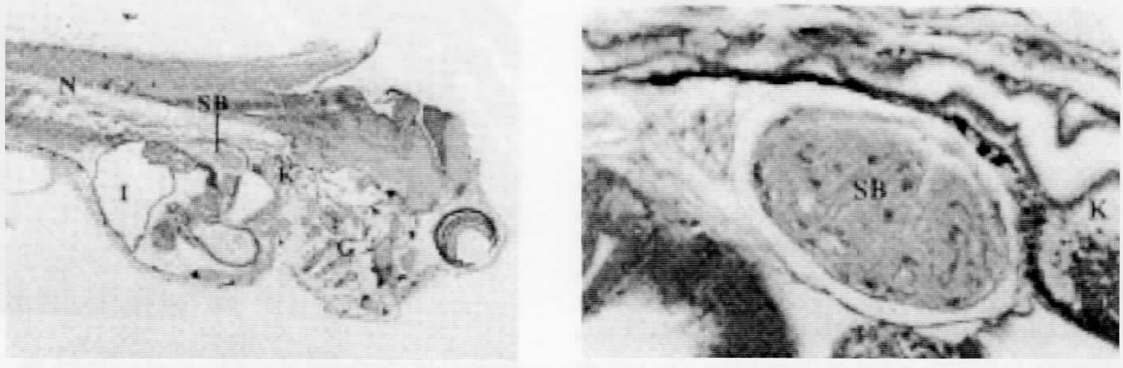

FIGURE 12. *Solea senegalensis* larvae 26DAH (A); Detail (B) SB - swim bladder; I - intestine; G - gills; N - notochord; K - kidney

related with the poor development of the olfactory and visual systems.

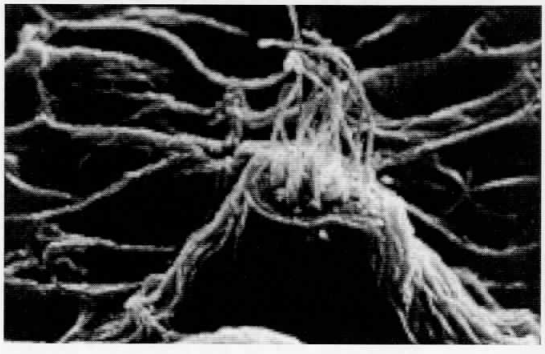

FIGURE 14. Neuromasts of *solea senegalensis* 17DAH (Scanning Electron Microscope SEM)

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