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Larvae digestion and new weaning experiments in *Solea senegalensis*

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SUMMARY – Senegalese sole (*Solea senegalensis*) is a common high-value flatfish in Southern Europe, commonly exploited in extensive aquaculture production in countries like Portugal and Spain. The interest in rearing *Solea senegalensis* has increased due to the decreasing market prices for sea bream and sea bass coupled with the need for aquaculture diversification. In spite of good results with broodstock management, the mass production of larval and juvenile fish of good quality has not yet been fully achieved. Nutritional aspects have been identified as major reasons for these results. However, despite the commercial interest there is a surprising lack of information on the biology, especially on the morphological and functional capacities of the digestive tract. This paper is a review of the last achievements to improve the conditions of rearing this species, such as the effect of background colour and light intensity on feeding efficiency and subsequent growth of *S. senegalensis* larvae, as well the major events which occur during the development of the larval digestive system. Although the major morphological events occur during the early stages, the gastric glands were observed only around 28 DAH (days after hatching). Alkaline and acid phosphates, lipase and aminopeptidase activities were observed from mouth opening onwards and increased with larval development. The development of enzymatic activities in brush border membranes suggested that intestinal maturation was initiated as early as 21 DAH in sole larvae. The data obtained suggests that only after 31 DAH *S. senegalensis* larvae have a morphologically complete digestive tract and they are able to digest and absorb complex nutrients. Preliminary results on growth of post-larvae of *S. senegalensis* fed with diets with different protein/lipid ratios were obtained. Growth rates in larvae fed with *Artemia nauplii* were up to 8.0% body weight/day. Larvae co-fed with *Artemia nauplii* and dry diets (1:1) with different protein/lipid ratios had growth rates up to 6.0% body weight/day. In the range of dietary protein/lipid ratios studied, higher ratios appear to have lead to an increased growth.

Key words: Sole, larviculture, digestive tract, digestive enzymes, weaning.

RESUME – *"Digestion chez les larves et nouvelles expériences de sevrage chez* Solea senegalensis*". La sole du Sénégal* (Solea senegalensis) *est un poisson plat commun, de haute valeur, du Sud de l'Europe, couramment exploité en production aquacole extensive dans des pays comme le Portugal et l'Espagne. L'intérêt de l'élevage de* Solea senegalensis *a augmenté en raison des prix de marché qui baissent pour la daurade et le bar parallèlement au besoin de diversifier en aquaculture. Malgré de bons résultats en gestion des reproducteurs, la production en masse de poissons larvaires et juvéniles de bonne qualité n'est pas encore complètement au point. Des aspects nutritionnels ont été identifiés comme étant les raisons majeures de ces résultats. Cependant, malgré l'intérêt commercial, il y a un manque surprenant d'information sur la biologie, spécialement sur les capacités morphologiques et fonctionnelles du tractus digestif. Cet article passe en revue les derniers acquis visant à améliorer les conditions d'élevage de cette espèce, tels que l'effet de la couleur de fond et l'intensité de la lumière sur l'efficacité alimentaire et par conséquent la croissance des larves de* S. senegalensis, *ainsi que les événements majeurs qui ont lieu pendant le développement du système digestif larvaire. Bien que les grands événements morphologiques surviennent pendant les premiers stades, les glandes gastriques n'ont été observées qu'à environ 28 jours après éclosion. Les activités des phosphates alcalins et acides, des lipases et aminopeptidases, ont été observées à partir du moment de l'ouverture de la bouche et ont augmenté avec le développement larvaire. Le développement des activités enzymatiques dans les membranes à bordure en brosse suggère que la maturation intestinale a été entamée dès 21 jours après éclosion chez les larves de soles. Les données obtenues suggèrent que ce n'est qu'après 31 jours post-éclosion que les larves de* S. senegalensis *ont un tractus digestif morphologiquement complet et qu'elles sont capables de digérer et d'absorber des nutriments complexes. Des résultats préliminaires ont été obtenus sur la croissance des post-larves de* S. senegalensis *recevant des régimes avec différents ratios protéines/lipides. Les taux de croissance chez les larves recevant* Artemia nauplii *ont été de jusqu'à 8,0% de poids corporel/jour. Les larves recevant en même temps* Artemia nauplii *et des régimes secs (1:1) avec différents ratios protéines/lipides avaient des taux de croissance de jusqu'à 6,0% de poids corporel/jour. Dans la gamme de ratios alimentaires protéines/lipides étudiés, les ratios les plus élevés semblent avoir mené à une meilleure croissance.*

Mots-clés : *Sole, larviculture, tractus digestif, enzymes digestives, sevrage.*

Introduction

Solea senegalensis is considered to have a high potential for the diversification of aquaculture due to the high market price, the possibility of reproduction in captivity and the reasonable results obtained during larval rearing (Dinis, 1992; Dinis and Reis, 1995; Magalhães and Dinis, 1996; Bedoui, 1997; Dinis *et al*., 1998).

In spite of good results with broodstock management (Dinis, 1992), the mass production of larval and juvenile fish of good quality has not yet been fully achieved (Dinis, 1992; Vásquez *et al*., 1994). Nutritional aspects have been identified as major reasons for these results. However, despite the commercial interest there is a surprising lack of information on the biology, especially on the morphological and internal development relating to functional capacities. Compared to adults, larval fish present different mechanisms concerning morphology, physiology, assimilation rates and digestion rates. Several authors (Dabrowski, 1984; Govoni *et al*., 1986; Segner *et al*., 1993; Sarasquete *et al*., 1995) report that the requirements of larvae and juveniles are different from those of adults. According to Segner *et al*., (1993, 1994) the knowledge of the physiology of nutrition during ongrowing provides necessary support for the understanding of larval nutritional needs. Moreover, present efforts to replace live food by inert microdiets depend largely on such knowledge (Bengtson *et al*., 1993; Yúfera *et al*., 1996). Indeed, *S. senegalensis* exhibits good growth during the larval period when fed live prey (Vázquez *et al*., 1994; Dinis and Reis, 1995); the weaning, switch from live prey to compound diet feeding, induces poor growth and mortality (Dinis, 1992; Marin-Magan *et al*., 1995).

According to Bengtson (1991), it is necessary to combine two types of studies: (i) the description of the development of structures and their functions; and (ii) the changes which have been induced by the food in structures and functions in order to understand and qualify the nutritional process, as well as the relationship between diets and growth during larval development. Furthermore, a better knowledge of the physiology of nutrition during ongrowing is then essential for the understanding of larval nutritional needs.

Successful culture of marine fish larvae depends also of many biological and physical aspects of the rearing systems. It is important that the rearing systems used to raise larvae of newly cultured species should be designed to avoid confounding variables. Most of the marine fish larvae are visual feeders and are dependent on a certain level of light to identify and attack the preys. As a result, feeding success depends not only on the quantity of suitable food but also on the visibility of the preys in the rearing tanks (Ina *et al*., 1979; Hunter, 1980).

This paper is a review of the last achievements to improve the conditions of rearing this species such as: (i) the effect of background colour and light intensity on feeding efficiency and subsequent growth; (ii) the major events which occur during the development of the larval digestive system; (iii) the effect of protein and lipid diet composition on growth and survival; and (iv) weaning into artificial diets.

Rearing conditions

Larvae of *Solea senegalensis* were obtained by natural spawning from a broodstock adapted to captivity at the University of the Algarve (Center of Marine Sciences, Portugal), and reared in 200 l conical tanks, from hatching 0 days after hatching (DAH) to 18 DAH after which they were transferred to 300 l flatbottom tanks until the end of the experiment (30 DAH). Larvae were reared under 13 hours light:11 hours dark dial cycle photoperiod. The oxygen concentration ranged between 6.8-7.2 mg/l, temperature between 18 to 25°C and salinity between 32 to 35‰. Larvae opened their mouth at 2 DAH and started to feed on rotifers (*Brachionus plicatilis* – small strain) at a density of 5 prey/ml. *Artemia nauplii* were introduced at 3 DAH and their density was gradually increased, becoming the only prey offered from 5 DAH onwards. Metanauplii was introduced when larvae were 12 DAH .

Growth

The larvae grew exponentially (Fig. 1) (R = 0.98). At hatching the mean length was 2.8±0.19 mm, reaching 10.0±1.20 mm by the end of the period studied.

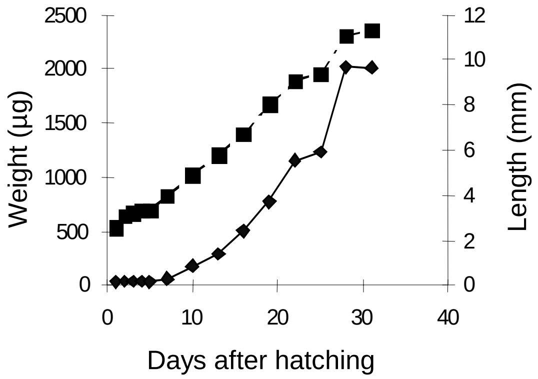

Fig. 1. Growth of *Solea senegalensis*: dry weight (solid line); total length (dashed line). Each mean [mean±SEM (n = 3)] is a pool of 15 larvae (Ribeiro *et al*., in press).

The relative growth rate was 14.47%/d ([exp ((In final weight – In initial weight)/(final day – initial day)) – 1*100]; Ricker, 1958) and 4.59%/d considering the length (Fig. 1).

Vision and larval rearing

Many larvae from marine species are visual predators and the appearance of the visual photoreceptores, the cones and the rods, are differs among species. The photoreceptors are responsible for the transformation of the light energy into the electric impulses, where the cones are responsible for diurnal vision and rods for nocturnal vision (Fernald, 1993). Feeding depends to a certain extent on the light intensity to identify and attack the preys, and is also related to the environmental condition of larval rearing.

In general, the epipelagic larvae have the retinal cells arranged radially and not yet differentiated. At this stage the cones and ganglionary cells are recognised. This means that the larvae need high light intensity levels in order to have sufficient vision.

Blaxter (1968) showed that marine fish larvae rely on visual stimuli to hunt and catch their prey. He reported that sole larvae eat in the dark although high feeding efficiency requires light, confirming the results of Rosenthal (1969) that sole larvae detected their prey by sight. When larvae migrate from the surface to deeper waters, the eyes become bigger and the rods appear, allowing the larvae to capture food in reduced light conditions. In conclusion, food and feeding are not the only factors to be considered in larvae rearing.

Furthermore, the conditions that affect the ability of larvae to see their feed items may affect the growth during larval development. Fonds (1979) considered that environmental factors such as turbidity may reduce the ability of sole larvae to select their planktonic prey. Dendrinos *et al*. (1984) found an improvement on feeding efficiency of Dover sole larvae (*Solea solea*) by artificially coloured *Artemia nauplii*.

Most of the common rearing larval tanks are black walled. These type of tanks give a good contrast between food and background and they were found to be suitable for rearing herring (Blaxter, 1968), turbot (Howell, 1979), dolphin fish (Ostrowski, 1989) and white bass (Denson and Smith, 1996).

Duray *et al*. (1996) reported that grouper larvae can be reared in both transparent or black tanks, but the use of algae in tanks increases the contrast with the rotifers and improves the visual perception of the preys.

In order to improve the conditions of rearing of *S. senegalensis* a study on the effect of background colour and light intensity on feeding efficiency (vacuity index and subsequent larval growth) was carried out (Aragão *et al.*, 1998). Experimental tanks were painted in white, orange and black in order to cover the normal black walled tanks commonly used in hatcheries, the orange tank with low contrast with *Artemia* and a white walled tanks for a good contrast with the preys and all of them submitted to different light intensities.

Results showed that a light intensity of 1200-1600 lux improved the feeding success of larvae in all tanks, although 500-850 lux appears to be beneficial to the larvae in white tanks too. In these tanks, larval exogenous feeding usually starts first, the vacuity index is lower and *Artemia* intake is higher than in other tanks (Table 1).

Table 1. Vacuity index and *Artemia* intake of *S. senegalensis* larvae at four light intensities, according to tank colour (Aragão *et al.*, 1998)

Light intensity (lux)	Tank colour	Exogenous feeding start	4th to 6th day		7th to 9th day	
			Vacuity index (%)	*Artemia* intake	Vacuity index (%)	*Artemia* intake
200-250	White	1st	100-20	0.0-1.6	70-0	1.2-4.0
	Black	2nd	100-33	0.0-1.1	100-10	0.0-3.6
550-850	White	1st	93-27	0.1-1.6	40-7	1.8-4.0
	Orange	3rd	96-65	0.0-1.1	80-50	0.6-3.4
	Black	2nd	100-85	0.0-0.3	86-47	0.3-1.8
1200-1600	White	1st	100-20	0.0-2.1	20-0	0.6-4.9
	Orange	3rd	100-56	0.0-0.7	50-0	0.6-3.2
	Black	2nd	100-30	0.0-1.3	50-10	1.3-3.0
1800-1900	White	1st	89-30	0.1-2.0	40-20	1.7-3.2
	Orange	2nd	100-60	0.0-1.1	60-10	1.1-3.0
	Black	3rd	100-90	0.0-0.1	100-60	0.0-0.8

Results also showed that both tank colour and light intensity affected the growth of *S. senegalensis* larvae (Table 2). However, there is no synergy between these two parameters.

Table 2. Mean dry weight of *S. senegalensis* larvae at four light intensities, according to tank colour

Light intensity (lux)	Tank colour	Mean dry weight (µg) at different ages			
		1 DAH	3 DAH	6 DAH	9 DAH
200-250	White	66	71	102	134
	Black	66	80	85	89
550-850	White	67	72	69	156
	Orange	67	72	67	119
	Black	67	78	83	108
1200-1600	White	66	80	84	117
	Orange	66	70	77	77
	Black	66	66	124	94
1800-1900	White	54	57	58	126
	Orange	54	61	60	109
	Black	54	51	55	90

Morphology of digestive tract

The major morphological changes of the digestive system of *S. senegalensis* larvae happen during the first 2 days after hatching, when larvae are exclusively dependent on endogenous reserves (Ribeiro *et al.*, 1999). During the rest of the studied period the changes observed were the appearance of gastric glands and the increase in size and complexity of the structures studied.

At hatching (0 day) the digestive tract was merely a tubular segment laying dorsally to the yolk sac and with both extremities closed.

Histologically we were able to differentiate: (i) 5 portions in the digestive tract (buccal-pharyngeal cavity, oesophagus, incipient stomach, anterior and posterior intestine); (ii) differentiation of mucosa epithelium, the opening of mouth and anus (Fig. 2); and (iii) the functionality of the liver and the pancreas (Ribeiro *et al.*, 1999). A similar sequence of changes was described for other flatfishes, *Solea solea* (Boulhic and Gabaudan, 1992) and *Paralichthys dentatus* (Bisbal and Bengtson, 1995) during the yolk-sac period, and also *Coregonus fera* (Loewe and Eckmann, 1988).

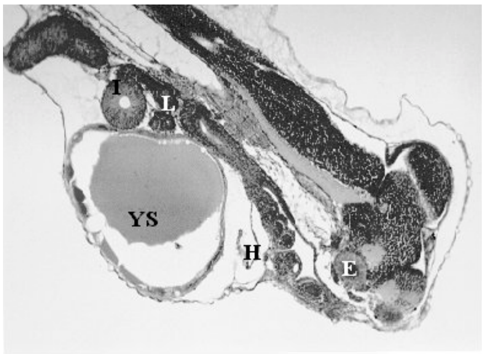

Fig. 2. Digestive tract of 2 DAH of *Solea senegalensis* larvae (paraffin section stained with Haematoxilin-Eosin). DS: digestive system; E: eye; H: heart; I: intestine; L: liver; OG: oil globules; YS: yolk sac.

The presence of mucous cells in the oesophagus on day 3 DAH, previously reported for *Solea senegalensis* (Sarasquete *et al*., 1996) and *Solea solea* (Boulhic and Gabaudan, 1992) and also the strong skeletic muscular layer coincides with the establishment of exogenous feeding. These mucous cells appear later in other species (Cousin and Baudin-Laurencin, 1986; Sarasquete *et al*., 1995). After 3 days of exogenous feeding, several vacuoles were observed in the anterior intestine, along with dense vesicles containing proteins in the posterior intestine and also an increase of vesicles (glycogen) in hepatocytes, indicating an increase in cell metabolism. The presence of vacuoles in the anterior intestine mucosa has been described in other species as an indicator of luminal absorption and storage of lipids (Iwai, 1969; Kjorsvik and Opstad, 1988; Sarasquete *et al*., 1995) during the larval phase due to poor lipid metabolism, which improves with larval growth (Loewe and Eckman, 1988).

At 19 DAH *Solea senegalensis* larvae achieved the benthic stage. At this point only an increase in the length and folding of the digestive tract was observed (Fig.3).

Although the incipient stomach is distinguished from day 3 (2 DAH) onwards, it is only around 28 DAH that we can observe the presence of gastric glands (Fig. 4). According to Tanaka (1971), the appearance of gastric glands indicates the beginning of the juvenile stage. Vu (1983), working with *Dicentrarchus labrax* described the differentiation of gastric glands around 25 DAH, but these only became functional when the fish were adults. Fish larvae without a functional stomach are known to have immature digestive mechanisms, especially in terms of protein digestion. This has been presented by several authors as one of the problems for the development of fish larvae diets (Dabrowski, 1984; Segner *et al*., 1994).

The analysis of data obtained by Ribeiro *et al.* (1999), suggests that after 31 DAH *S. senegalensis* larvae are capable of ingesting, digesting and absorbing nutrients, having a morphologically complete digestive tract.

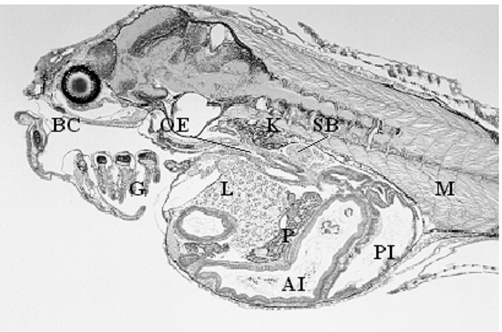

Fig. 3. Digestive tract of 19 DAH of *Solea senegalensis* (paraffin section stained with Haematoxilin-Eosin). AI: anterior intestine; BC: buccal cavity; E: eye; G: gills; GG: gas gland; K: kidney; L: liver; M: muscle; N: notochord; OE: oesophagus; P: pancreas; PI: posterior intestine; SB: swim bladder.

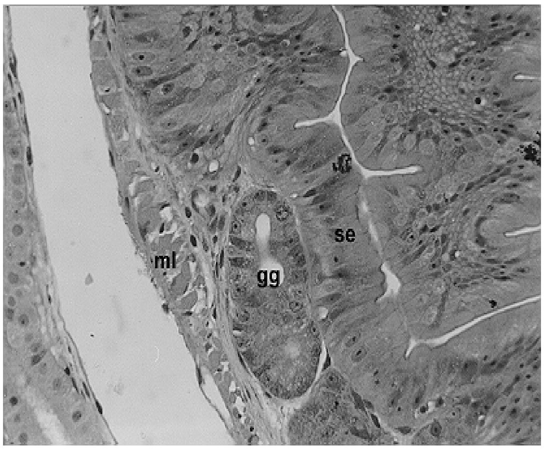

Fig. 4. Gastric glands in 30 dah *Solea senegalensis* larvae (metacrylate section stained with Toluidine Blue). ML: muscular layer; GG: gastric glands; SE: stomach epithelium.

Digestive enzymology

With the end of the endogenous nutritional reserves, marine fish larvae must obtain food in order to have energy for their maintenance metabolism and for growth (synthesis of new tissues). Obtaining energy from the food means capturing, ingestion, digestion, absorption and assimilation.

Through histological analysis it could be verified that, in general, at mouth opening marine fish larvae have a differentiated digestive tract. This indicates that they are able to ingest and digest food. However, the positive identification of a digestive capacity implies the presence of the digestive enzymes that will act on the different nutrients (proteins, lipids and carbohydrates).

Digestive enzymes belong mainly to the group of hydrolases (Table 3) and are distributed along the digestive tract. The nutrient digestion starts in the mouth (amylase, carbohydrates), continues in the stomach where the enzymes function at a pH lower than 2.0 (pepsin, proteins). Finally, digestion is finalised in the intestine, with the contribution of the pancreas, gall bladder and liver (pancreatic amylase, carbohydrates; pancreatic and enteric lipase, lipids; trypsin, chimotrypsin and aminopeptidases, proteins) and the brush-border enzymes (peptidases, phosphatases).

Leucine-alanine-peptidase exhibited two significantly different levels of specific activity until 19 DAH. A high level of specific activity until 7 DAH, strongly decreasing to a level 6 times lower than the earlier stages (Fig. 5).

Table 3. Digestive enzymes organ of synthesis place of action and substrates

Enzymes	Synthesis	Place of action	Substrate
Trypsin, chimotrypsin	Exocrine pancreas	Anterior intestine	Peptides
Amylase	Exocrine pancreas	Anterior intestine	Carbohydrates
Lipase	Exocrine pancreas		Lipids
Aminopeptidase	Brush-border		Dipeptides
Peptidases	Intestinal epithelium		Peptides
Alkaline phosphatase	Brush-border		Phosphor esters
Acid phosphatase	Brush-border		Proteins
Pepsin	Gastric glands	Stomach	Proteins

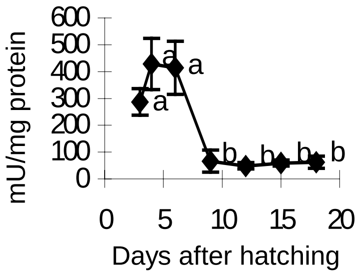

Fig. 5. Specific enzymatic activity of a cytosolic enzyme leu-ala; means±SD (A, n = 2; B, n = 3) with the same superscript are not significantly different ($p < 0.05$). Ribeiro *et al.* (in press)

The results of Ribeiro *et al.* (in press) showed that Senegal sole larvae exhibited enzymatic activity before first feeding. After a decreased in the specific activity of trypsin during the first days, a strong increase was observed after 4 DAH reaching the highest value on 9 DAH, after what a decrease was observed until the end of the period studied (Fig. 6A). Amylase exhibited a similar pattern of variation of specific activity along this period (Fig. 6B). The variation of the specific activity during early stages followed by a decrease to a constant level is characteristic of developing vertebrates (Henning, 1987) including fish (Cahu and Zambonino-Infante, 1994).

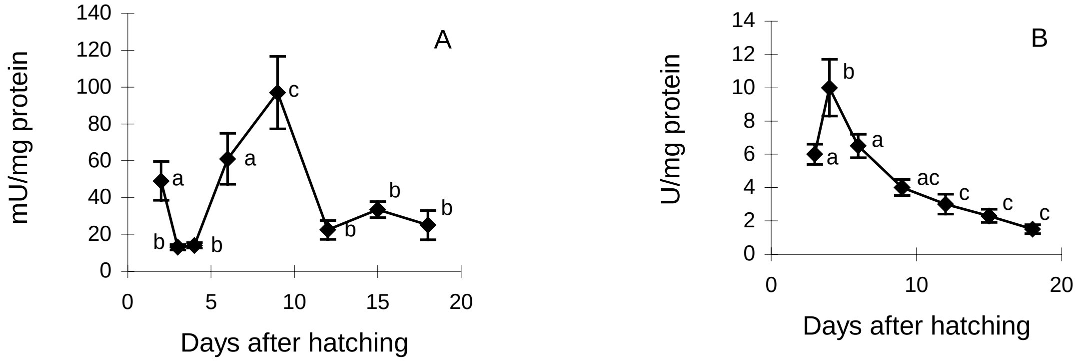

Fig. 6. Specific activity of trypsine (A) and amylase (B) in whole body homogenate.

The decrease in cytosolic activity suggests a decline in the pynocitotic digestion (Ribeiro *et al.*, in press), which can be in agreement with the maturation of the intestine induced by the pattern observed in the specific activity of trypsin and amylase.

The dissection of the digestive tract in larvae older than 19 DAH allowed us to observe an increase in the activity of the brush border enzymes (Fig. 7A) with a concomitant decrease in the cytosolic enzymes (Fig. 7B).

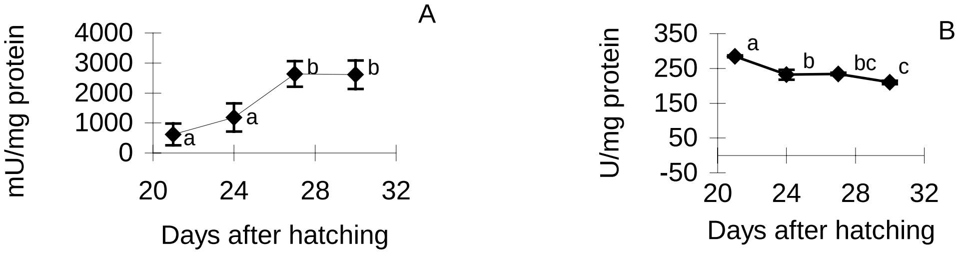

Fig. 7. Specific enzymatic activity of two intestinal enzymes in dissected larvae: a brush border membrane enzyme, alkaline phosphatase (A); a cytosolic enzyme, leu-ala (B). Means±SD (A, n = 2; B, n = 3) with the same superscript are not significantly different ($p < 0.05$).

In conclusion, it seems that from 25 days old onwards, larvae acquire an adult way of digestion (Ribeiro *et al*., in press).

Diet composition growth and survival

Growth and survival are primarily dependent on an adequate intake of nutrients. Proteins and lipids are the two main dietary components. Compliance with both quantitative and qualitative (essential amino acids, essential fatty acids) requirements of these nutrients are paramount. The dietary protein/lipid ratio is also believed to be an important determinant of diet utilisation (Bromley, 1980; Andersen and Alsted, 1993). Low protein/lipid ratios in the diet may lead to depressed growth, which may have consequences on growth and survival in later stages, while diets with a high protein/lipid ratio may result in a low conversion efficiency of protein (Conceição, 1997), the most costly diet component. However, little is known about the optimal protein/lipid ratios in larval and post-larval marine fish.

In order to study the effect of different protein/lipid ratios on growth of *Solea senegalensis* post-larvae were fed *Artemia* enriched for 24 h with a mix of the algae *Tetraselmis* sp. and *Isochrysis* sp. or a commercial lipid emulsion – Super Selco (INVE Aquaculture, Belgium) – between 36 and 63 DAH (Conceição *et al*., unpublished results). The results suggest that growth rates and survival are higher (Figs 8 and 9) for post-larvae fed *Artemia* enriched with algae (17% total lipid, protein/lipid ratio 3.4) when compared to the ones fed *Artemia* enriched with Super Selco (32% total lipid, protein/lipid ratio 1.4). Total lipid contents of the larval muscle were also higher in the Super Selco-enriched treatment (Fig. 10). Therefore a high protein/lipid ratio in the diet may lead to improved growth rates in sole post-larvae, and enrichment of *Artemia* with oil emulsions may be sub-optimal.

Weaning

Adaptation of marine fish larvae into artificial diets is a critical period were large mortalities often occur (Watanabe and Kiron, 1994). Cultivation of sole post-larvae is in this respect particularly difficult and generally characterised by high mortality (Dinis, 1992).

Following the previously referred experiment of Conceição *et al.* (unpublished results), sole post-larvae were slowly weaned to two different artificial diets, the ICES standard weaning diet (produced by the Lab. of Aquaculture & ARC, University of Gent, Belgium) and a INVE test diet for sole (INVE Aquaculture, Belgium) from 63 to 91 DAH, and reared further until 120 DAH. A third group was fed *Artemia* metanauplii of 48 h, fed on a mix of algae *Tetraselmis* sp. and *Isochrysis* sp. The results show that independently of the diet type and composition (see Fig. 9) survival in any of the treatments was less than 5% between 36 and 120 DAH. A large part of the mortality in the groups fed with the artificial diets was verified during the period of co-feeding with *Artemia*. Furthermore, the mortality in the *Artemia* treatment was comparable to the artificial diets. Mortality was high in this experiment compared with previous results (Bruzon *et al*., 1995; Esteban *et al*., 1995; Marin Magan *et al*., 1995; Rodriguez *et al*., 1995; Dinis *et al*., unpublished), probably due to the much smaller size of the rearing units, and the absence of natural food organisms.

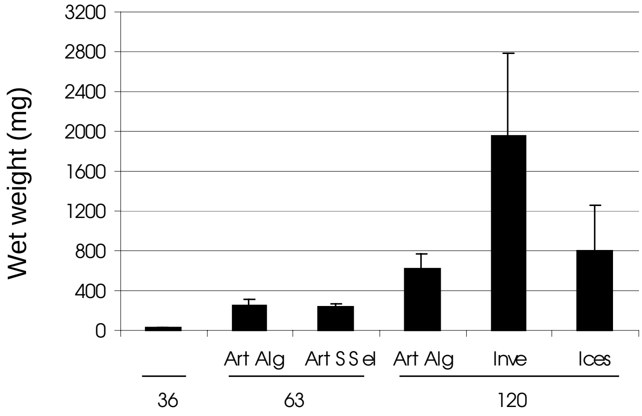

Fig. 8. Wet weights of *Solea senegalensis* post-larvae at 36, 63 and 120 days after hatching (DAH) after the experiments of Conceição *et al*. (unpublished results). Post-larvae were fed *Artemia* with algae (Art Alg) or Super Selco (Art SSel) between 36 and 63 DAH. From 63 to 91 DAH post-larvae were slowly weaned to the ICES and the INVE diets, or fed *Artemia* metanauplii of 48 h raised on algae.

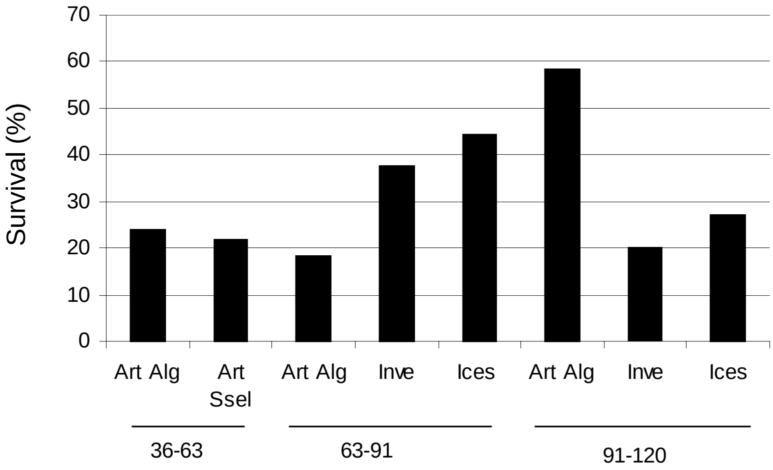

Fig. 9. Survival of *Solea senegalensis* post-larvae during the different phases of the experiments of Conceição *et al.* (unpublished results). Treatments and details as in Fig. 8.

The common high mortalities in *Solea senegalensis* may be related to sub-optimal or fluctuating environmental factors (e.g., temperature and salinity), inadequacies in the rearing systems used due to the sudden transition of the initially pelagic nature to a benthic one, deficiencies in the previous nutritional history (larval stage), and an eventual poor egg quality.

Sole post-larvae fed with the artificial diets may grow better than the *Artemia*-fed animals (see Fig. 8). The final weights of the fish are in the range of what previously observed for sole of about the same age reared in large volumes (Esteban *et al*., 1995; Rodriguez *et al*., 1995).

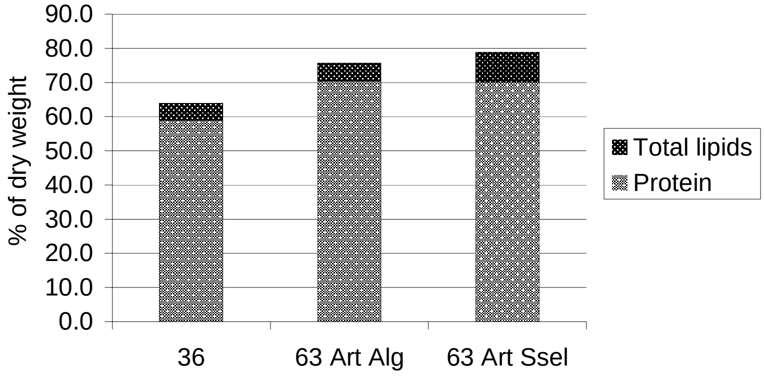

Fig. 10. Total lipid (analysed by modified Bligh and Dyer method) and total protein (analysed by Lowry method) contributions to muscle protein dry weight in *Solea senegalensis* post-larvae fed *Artemia* enriched for 24 h with a mix of the algae *Tetraselmis* sp. and *Isochrysis* sp. (Art Alg) or Super Selco (Art SSel) between 36 and 63 DAE (from Conceição *et al*., unpublished results).

Conclusions

Feeding efficiency is related to the colour of the tanks and light intensity, and care should be taken in order to provide an adequate contrast between prey and tanks.

The major morphological changes of the digestive system of *S. senegalensis* larvae happen during the first 2 DAH when the alimentary canal is subdivided into functionally different regions.

Gastric glands were observed around 28 DAH.

Senegal sole larvae exhibited enzymatic activity at first feeding.

Variation in specific activity during early stages decreasing to a constant level, suggests intestinal maturation.

A high protein/lipid ratio in the diet may lead to improved growth rates in sole post-larvae.

It seems to be possible to wean sole post-larvae with artificial diets.

The use of the artificial diets does not seem to be the main factor leading to the high mortalites commonly verified during weaning of sole.

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