

Nutritional physiology during development of Senegalese sole (*Solea senegalensis*)

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Abstract

The Senegalese sole, a species with a complex metamorphosis, difficulties in weaning and with occasional problems of malpigmentation and skeletal deformities, is a good model species to study larval nutritional physiology. In addition, the early metamorphosis and acquisition of a peculiar non-proactive bottom-feeding behaviour make early weaning an important issue in sole hatcheries. The present work reviews recent findings in different aspects of nutritional physiology during the development of Senegalese sole, in an attempt to optimize the composition of sole diets and to understand what are the limiting factors for weaning sole. Both digestive enzymes activity and tracer studies using ^{14}C -*Artemia* show that sole larvae, even at young stages, have a high capacity for digesting live preys. This is reflected in a high growth potential and low mortality rates for this species during the larval stage compared to other marine fish species. Based on the observation of the digestive enzymes profile, early introduction of inert microdiets in co-feeding with *Artemia* does not seem to affect intestinal function. However, when co-feeding is not provided, intestinal activity may be depressed. Furthermore, early introduction of microdiets in co-feeding with *Artemia* may have a positive effect on survival rates, but at the expense of lower growth rates and higher size dispersal. This may reflect variation in the adaptation capacity of individual larvae to inert diets. High dietary neutral lipid (soybean oil) content results in reduced growth and accumulation of lipid droplets in the enterocytes and affects the capacity of Senegalese sole larvae to absorb and metabolise dietary fatty acids (FA) and amino acids (AA). Through tube feeding of different ^{14}C -lipids and free FA it has been shown that FA absorption efficiency increases with unsaturation and that sole larvae spare DHA from catabolism. In addition, it was demonstrated that absorption efficiency varies according to molecular form, being highest for free FA, lowest for triacylglycerols and intermediate for phospholipids. Live preys commonly used in larviculture do not seem to have a balanced AA profile for sole larvae. Furthermore, the ideal dietary AA composition probably changes during development. Rotifers and *Artemia* metanauplii are apparently deficient in one or more of the following AA depending on the larval development stage: histidine, sulphur AA, lysine, aromatic AA, threonine and arginine. It has also been demonstrated that balancing the dietary AA profile with dipeptides in *Artemia*-fed larvae increases AA retention and reduces AA catabolism. When supplementing larval diets with limiting AA it should also be considered that sole larvae have different absorption, and retention efficiencies for individual AA, and that they have the capacity to spare indispensable AA. In addition, the absorption of free AA is faster and more efficient than that of complex proteins. Improvements in biochemical composition of inert microdiets for sole are likely to contribute to the reproducible weaning success of Senegalese sole.

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1. Introduction

Sole is considered a promising candidate for marine aquaculture in Europe since the nineties (Howell, 1997; Dinis et al., 1999). However, with recent decrease in profit margins for the two main cultured species in Southern Europe, gilthead seabream and European sea bass, interest in sole culture has boosted. Two species are of interest, the Dover sole (*Solea solea* Linnaeus, 1758), mainly in the Atlantic coast, and Senegalese sole (*Solea senegalensis* Kaup, 1858) in the Mediterranean and South-Atlantic areas (Imsland et al., 2003).

The Senegalese sole, a species with a complex metamorphosis (Ribeiro et al., 1999a; Fernández-Díaz et al., 2001), difficulties in weaning, occasional problems of malpigmentation (Soares et al., 2002; Villalta et al., 2005a), and high incidence of skeletal deformities (Gavaia et al., 2002; Engrola et al., 2005), is a good model species to study larval nutritional physiology. Qualitative and quantitative dietary imbalances are one of the main causes of sub-optimal performance and quality often observed in larviculture.

Weaning has traditionally been a bottleneck in sole culture (Howell, 1997; Dinis et al., 1999). Still, in recent years improvement of zootechnical conditions and especially technological improvements in inert diets, including the development of “soft” microagglomerated products containing protein hydrolysates (Day et al., 1997, 1999), allowed solving these weaning difficulties for Dover sole (Howell, 1997; Day et al., 1999). Progress was also substantial for Senegalese sole, but reproducibility of good weaning results is still a problem (Engrola et al., 2007). Weaning seems to be more difficult in Senegalese sole. In addition, the early metamorphosis and acquisition of a peculiar non-proactive bottom-feeding behaviour make early weaning an important issue in sole hatcheries.

The current knowledge of sole biology and ecology, and the state of development of its culture in captivity, has been recently reviewed and synthesized (Imsland et al., 2003). The present work reviews recent findings in different aspects of nutritional physiology during the development of Senegalese sole larvae and post-larvae, in an attempt to optimize the composition of sole diets and to understand which are the limiting factors for weaning sole.

2. Rearing conditions

2.1. Larval rearing

Senegalese sole eggs are normally obtained from naturally spawning wild caught broodstock (Dinis et al.,

1999; Imsland et al., 2003). Incubation takes place at 18–20 °C, normally in cylindro-conic incubators, and slight aeration and water renewal are provided during the whole incubation period.

Once hatched, larvae are stocked into larval rearing tanks at a density of 30 to 100 larvae/L. Tank volume varies from 70 to 700 L or more and is usually of a cylindro-conical or cylindrical shape (Cañavate and Fernández-Díaz, 1999; Ribeiro et al., 1999a; Morais et al., 2004a). Light intensity of 1200 lx at the surface or more results in improved feeding success, although good results may be obtained above 500 lx if white colored tanks are used (Dinis et al., 2000). A photoperiod ranging from 16 h light:8 h dark to continuous is commonly used. Larval rearing takes place at temperature ranging from 18 to 20 °C and using a salinity of 33 to 35‰ (Dinis et al., 1999). Green water technique is a common practice during larval rearing using either a mixture of *Isochrysis galbana* and *Tetraselmis chui* (Ribeiro et al., 1999a) or *Nannochloropsis gaditana* (Fernández-Díaz et al., 2001).

Commonly, enriched *Brachionus* sp. are offered from mouth opening until 4 days after hatching (DAH). Small strain newly hatched *Artemia* nauplii are introduced at 4 DAH and stopped at 9 DAH. Still, mouth gap of sole larvae at first feeding is sufficiently wide to allow direct feeding on small strain newly hatched *Artemia* nauplii. Normally a rotifer feeding period is introduced mainly to guarantee higher levels of highly unsaturated fatty acids (HUFA) in the diet. In this respect, the rotifer feeding period may also be extended until 9 DAH (Cañavate and Fernández-Díaz, 1999). However, feeding plans without rotifers have been used with success for rearing Senegalese sole larvae. Enriched *Artemia* metanauplii are included in the feeding regime at 9 DAH and quantity gradually increased. Around 12 DAH larvae metamorphosis is strongly accelerated and the first animals leave their pelagic lifestyle. Enriched *Artemia* metanauplii can then be gradually offered under a frozen form allowing an easier catch to the benthic larvae. End of larval rearing coincides with the settlement of all larvae and usually occurs around 19 DAH (Dinis et al., 2000; Engrola et al., 2005).

2.2. Post-larval rearing

Post-larvae are generally transferred when benthic stage is achieved around 19 DAH (Dinis et al., 1999) to flat bottom tanks with 2–10 cm water column (Ribeiro et al., 2002; Engrola et al., 2005). Fish are reared in an open, closed or semi-closed system at a density of 3000 fish/m² until weaning is completed. A photoperiod of 12 h light:12 h dark with a light intensity

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