Aquaculture 496 (2018) 1–9

Contents lists available at ScienceDirect

Aquaculture

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

Review

Towards an early weaning in Senegalese sole: A historical review

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ARTICLE INFO

Keywords:
Senegalese sole
Weaning
Microdiet
Artemia replacement
Co-feeding

ABSTRACT

Senegalese sole (*Solea senegalensis*) is a highly valuable flatfish species targeted for aquaculture diversification in Southern-European countries. Although its adaptation to captivity was foreseen in the 1970´s, its commercial production at a relevant industrial scale is extremely recent. One of the factors causing this delay in commercialisation was a severe bottleneck occurring during weaning, where incipient larval growth performances and survivals were impairing farming during the first decades of farming. However, a significant evolution has recently occurred for Senegalese sole weaning, with larval growth rates, survivals and quality drastically increasing, and good results becoming more reproducible. These progresses were only possible following significant R&D efforts leading to increased knowledge on larval nutritional physiology, weaning strategies, feeding practices and microdiet formulation and production. To this end, this work reviews the advancements that contributed for the evolution occurring in Senegalese sole weaning during the last decades. This overview shows that, for instance, while in the year 2000 larval weaning would only be achieved at around 60 days after hatching (DAH), with fish achieving 1 g (wet weight basis) at around 120 DAH, nowadays it is possible to perform an early weaning starting immediately after settling (15 DAH) and achieving 1 g at 65 DAH. Future perspectives on Senegalese sole weaning should target an optimisation of microdiet nutritional and physical properties to a further adequacy for the pelagic larvae, where the predatory capacity is reduced and lower particle sizes lead to fast and high nutrient leaching. Nevertheless, this work shows that early life-feed replacement is a reality for Senegalese sole, although it remains uncertain if it will ever occur at the onset of exogenous feeding.

1. Introduction

The global demand for seafood strongly increased during the last 20 years, leading the aquaculture industry to rapidly increase its production, becoming the fastest growing sector in the animal production field. In 1990, aquaculture contributed with around 25% of total world fisheries, while in 2014 these values reached around 45% (FAO, 2016). Nevertheless, aquaculture production strongly grown in China and other Asian countries due to a boost on the farming of freshwater fish species, but it decreased or stagnated in European countries, with Europe reducing its global production share from 12% in 1990´s to 4% in 2012. Currently, European aquaculture is dominated by few farmed species that are facing market saturation: Atlantic salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). For this purpose, diversification of cultured species resulted as an outcome of EATIP's strategic agenda as a main strategy to boost European fish farming, with project DIVERSIFY (7FP-KBBE-2013-GA 602131; 2013–2018) focusing on potential of candidate species for industrial farming expansion including meagre (*Argyrosomus regius*), greater amberjack (*Seriola dumerili*), wreckfish (*Polyprion americanus*), Atlantic halibut (*Hippoglossus hippoglossus*), grey mullet (*Mugil cephalus*) and the pikeperch (*Sander lucioperca*).

In Southern Europe, the listing of interesting species for aquaculture diversification also includes Senegalese sole (*Solea senegalensis*), a flatfish species with a high quality white flesh and market value, currently reaching around 12 to 14 € kg^{-1} at farm gate. Although Senegalese sole started its adaptation to captivity in the 1970s, its industrial production has only recently taken-off, being an achievement of many years of research and technological development. While the production of Senegalese sole was negligible in 2009, its foreseen production for 2017 will reach over 1600 tons (APROMAR, 2017). The reasons for the delay in Senegalese sole industrial farming were mostly related to a high vulnerability to diseases as Pasteurellosis or Flexibacteriosis, difficulties in reproducing the G1 broodstock (fish that undergo a complete life-cycle under captivity conditions) and difficulties in the transition from live-feeds onto formulated diets (weaning).

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https://doi.org/10.1016/j.aquaculture.2018.06.077
Received 30 December 2017; Received in revised form 4 April 2018; Accepted 25 June 2018
Available online 04 July 2018
0044-8486/

Newly-hatched Senegalese sole larvae are pelagic with bilateral symmetry, undergoing an extremely accentuated metamorphosis at 10 to 20 DAH, a process that achieves its climax around 14 to 17 DAH. During this stage, the left eye migrates towards the right side and a 90° rotation in the body position occurs (Dinis et al., 1999; Fernández-Díaz et al., 2001). During this stage, the internal organs are rearranged and the digestive tract is reorganised, with migration of the anus towards the pelvic fin (Ribeiro et al., 1999). This process results in a benthonic postlarva that lies settled on the blind side. The exogenous feeding starts at 2 DAH, and although larvae can feed upon *Artemia* nauplii at this stage, rotifers are usually the first prey during few days (Conceição et al., 2007). Although Senegalese sole larvae exhibit an excellent growth performance and survival while feeding upon live-feeds, increasing live-feed replacement by inert microdiets during the early developmental stages would bring obvious benefits for the husbandry of the species. Namely, inert microdiets provide nutritional consistency and off-the-shelf availability, whereas live-feeds may act as vectors for diseases, have a variable nutritional value, their nutritional quality is difficult to manipulate and they are costly and time-consuming to produce (Hart and Purser, 1996). However, weaning has traditionally been considered a major bottleneck for Senegalese farming, justifying a significant amount of research conducted during the last decades to improve larval performance during this stage, as well as to perform an earlier transition from live-feeds to microdiets. A strong R&D effort lead the performance of Senegalese sole during weaning to greatly progress in the last decades, mostly due to recent advances in larval husbandry, feeding regimes and microdiet quality.

This work aims at reviewing progress made in Senegalese sole weaning during the last decades, as well as providing insights on weaning strategies and microdiet quality leading to such advancements. Furthermore, this work aims at demonstrating the latest R&D efforts to achieve an earlier weaning in Senegalese sole while maintaining high larval quality, growth performance and survival.

2. 1990 to 2004: early efforts on Senegalese sole weaning and increasing knowledge on larval nutritional physiology

Initial efforts on Senegalese sole weaning report to the early 1990s (Dinis, 1992). At this time, limited information existed regarding the biology of the species during the early developmental stages. Therefore, comparing performance results with a more established species with a similar life-cycle such as the Dover sole (*Solea solea*) were unavoidable. In Dinis (1992), Senegalese sole with 25 mg (wet weight basis) were cofed *Artemia* and rehydrated pellets (microdiet) from 31 to 40 days after hatching (DAH), feeding on the pellets alone until 131 DAH. Although the author considered the rehydrated pellets to contain a satisfactory nutritional quality, at the end of 100 days of trial only 36% of the fish survived. Furthermore, relative growth rate values (RGR) for this period were extremely low (1.7% per day; Table 1). These weaning results were far below those obtained for the Dover sole during the 1980´s, where RGR values between 5 and 7% per day were obtained, with survival ranging from 40 to 85% (Gatesoupe and Luquet, 1982; Métailler et al., 1983). Still, Dinis (1992) initiated weaning of Senegalese sole with a post-larval weight 5-fold lower to those used for the Dover sole in the study by Métailler et al. (1983), where highest survival values were achieved. Based on these differences, Dinis (1992) suggested that Senegalese sole weaning would be more successful if performed at a larger size.

Seven years following the study by Dinis (1992), Cañavate and Fernández-Díaz (1999) performed an evaluation of different weaning strategies for Senegalese sole using a commercial microdiet. Results showed that among the different weaning strategies adopted, the best results were obtained when a co-feeding regime with *Artemia* and a commercial diet was adopted from mouth opening until 50 DAH. In this regime, live-feed was reduced in a transition period from 43 to 49 DAH, with fish feeding on microdiets alone from 50 to 70 DAH. This strategy resulted in a very positive relative growth rate (10.3% per day) during the experimental period, although survival following the transition period (43 to 70 DAH) was relatively low. Moreover, Cañavate and Fernández-Díaz (1999) also showed that a sudden-weaning strategy at the onset of exogenous feeding would lead to an extremely low survival (1% at 23 DAH) and incipient growth performance. These findings clearly indicated that at this time the use of a commercial diet alone from mouth opening was unable to sustain growth and development of Senegalese sole larvae.

Ribeiro et al. (1999) described the ontogeny of the digestive system of Senegalese sole, with respect to digestive enzymes and distribution of lipids, carbohydrates and protein in different tissues during the first month of life. Such studies had been previously conducted for different species, such as Turbot (*Scophthalmus maximus*) (Cousin and Baudin Laurencin, 1985), Atlantic cod (*Gadus morhua*) (Kjorsvik et al., 1991), Fera (*Coregonus fera*) (Loewe and Eckmann, 1988), Dover sole (Boulhic and Gabaudan, 1992), Summer flounder (*Paralichthys dentatus*) (Bisbal and Bengtson, 1995), gilthead seabream (*Sparus aurata*) (Sarasquete et al., 1993; Sarasquete et al., 1995) and yellowtail flounder (*Pleuronectes ferrugineus*) (Baglole et al., 1997). In fact, a first approach had already been performed for Senegalese sole, from yolk-sac stage and until 15 DAH (Sarasquete et al., 1996). Ribeiro et al. (1999) revealed that Senegalese sole possesses an incipient stomach from 2 DAH onwards and that the appearance of gastric glands only arises at 27 DAH. These findings were later confirmed by Dinis et al. (2000), who observed the appearance of gastric glands in Senegalese sole at 28 DAH. The absence of a completely functional stomach at the first weeks of exogenous feeding indicated a lower capacity for larvae to digest complex proteins present in microdiets, justifying the unsuccessful results obtained by Cañavate and Fernández-Díaz (1999) when a sudden-weaning strategy was adopted at this stage. Nevertheless, Ribeiro et al. (1999) and Martinez et al. (1999) also observed relevant activities of alkaline and acid phosphatase, lipase and aminopeptidase in Senegalese sole from the mouth opening onwards, and increasing with larval development. The presence of aminopeptidase activity in the digestive tract so early in development suggested that the metabolism of small peptides would be ensured by pancreatic digestive enzymes, as foreseen for African catfish by Verreth et al. (1992).

Rønnestad et al. (2000) conducted a first report on the kinetics of amino acid (AA) absorption during the early life stages of marine fish, using 23 DAH Senegalese sole. In this study, which ultimately aimed at providing further knowledge to improve microdiet formulation for marine fish larvae, the assimilation rates of free amino acids (FAA) and protein were determined. Results showed that a FAA-based diet was assimilated faster (3.5 ×) and with a greater efficiency (80% vs 58%) than a protein-based diet. The authors concluded that FAA would potentially be a better dietary source of amino acids for Senegalese sole post-larvae. Yet, the authors also highlighted that care should be taken when using both protein and FAA sources, whereas differences in the absorption dynamics could lead temporary amino acid imbalances and increased catabolism. These findings partially explained some of the historical difficulties in the adaptation of Senegalese sole larvae to microdiets, supporting the need to include a fraction of dietary AA in a simpler molecular form. Interestingly, in a subsequent study, Rønnestad et al. (2001) showed that Senegalese sole post-larvae could even discriminate between dispensable and indispensable AA, preferentially utilising the former for energy production and the latter for body protein deposition (growth). These findings were later confirmed by Aragão et al. (2004a), using a tube-feeding method with Senegalese sole post-larvae, that balancing the dietary AA profile would increase AA retention, improving growth potential and nitrogen utilisation.

Several studies highlighted the importance of improving the adequacy of nitrogen sources and respective molecular weight profile in weaning microdiets for Senegalese sole (Ribeiro et al. (1999), Rønnestad et al. (2000), Rønnestad et al. (2001)). Still, it was necessary to wait for two years following the publication by Rønnestad et al.