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Stocking density and sex influence individual growth of Senegalese sole (*Solea senegalensis*)

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ABSTRACT

Growth is usually inversely correlated with stocking density of fish in culture. Senegalese sole aquaculture is affected by a high size variability and thus, this work tried to investigate the relationship of growth with density of two populations of 96 individually tagged Senegalese sole (318.7 ± 7.9 g; mean ± standard error of the mean). Fish were reared at low (LD) and high (HD) density (60% and 180% of bottom coverage respectively) for 195 days. After 134 days (period 1), density conditions were exchanged between groups. Mean weight, standard length, maximum width and centroid size were calculated for each of the 11 census days of the experiment. White muscle biopsies were taken in 7 of the census days in order to assess the RNA/DNA ratio, as a biochemical indicator of growth. Stocking density had an important effect on growth, as fish reared under HD showed poor or no growth during a 'lag phase' on the first 61 days of the experiment, leading to a significantly lower specific growth rate (0.23 ± 0.014) for period 1 compared with LD fish (0.34 ± 0.016). Fitting of linear mixed-effects (LME) models for the first 134 days of experiment showed a significant effect of density and sex on all the assessed biometric parameters. These results could be attributed mainly to the first 61 days of the experiment, as no differences were observed between days 61 and 134 in all the measurements, except for standard length, that showed to be lower for HD fish throughout the whole period. Fish reared under high density tended to grow slower than fish held at low density, while females showed faster growth than males, particularly in HD. Nevertheless, due to high size variability, no significant differences could be found in the mean values of weight or standard length after 134 days (467.2 ± 21.6 g and 28.7 ± 4.2 cm; 502.6 ± 22.5 g and 29.7 ± 4.5 cm for HD and LD fish respectively). Size variability could be an indicator of the onset of hierarchies, being stronger and with more females as dominant individuals than males in HD. After exchanging densities, and up to day 195, a similar lagging effect could be observed in LD fish exposed to high density, suggesting that a sudden change in density, more than density itself, could be the responsible for a detrimental effect on growth. RNA/DNA ratios, were significantly lower for HD fish between days 20 and 61.

1. Introduction

Senegalese sole has been the subject of thorough research in the last two decades, because of its high market demand, high market value and the adaptability of existent facilities to accommodate its rearing (Imsland et al., 2003). These facts present sole as an interesting new species for diversifying Mediterranean marine aquaculture.

Consumers purchase as sole indistinctly *Solea solea* or *S. senegalensis* (Reig et al., 2000), and different results have been obtained to date in terms of reproduction, growth or, in general, success in the consistent and reliable supply of farm-reared sole of both species to the market. Traditionally, Southern Europe countries have been more focused in *S. senegalensis* aquaculture among both species of sole, due to the lower spawning temperature requirements of *S. solea* (Howell, 1997) and the high abundance of *S. senegalensis* in Mediterranean and Southern Atlantic waters (Dinis et al., 1999), the former being nowadays the only sole species reared in Spain or Portugal.

Following some promising trials in early 80s, rearing of *S. senegalensis* has succeeded in key points such as reproduction (Anguis and Cañavate, 2005), weaning (Cañavate and Fernández-Díaz, 1999; Engrola et al., 2009), or nutrition (Rønnestad et al., 2001; Aragão et al., 2003; Morais et al., 2006; Conceição et al., 2007). However, diverse growth performance and high size variability is still an important issue when rearing Senegalese sole in captivity (Dinis et al., 1999; Flos et al., 1995, Flos et al., 2001; Rueda-Jasso et al., 2004).

Stocking density has been demonstrated as a crucial variable regarding growth performance of cultured fish. The effects of density on growth are diverse, usually showing a negative correlation in several finfish species as rainbow trout (*Oncorhynchus mykiss*) (Refstie 1977), Atlantic cod (*Gadus morhua*) (Lambert and Dutil, 2001) or in flatfish species like turbot (*Scophthalmus maximus*) (Irwin et al., 1999). Although, it has been noted that too low densities can also have a

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negative effect on growth in species that present schooling behavior as Arctic charr (*Salvelinus alpinus*) (Jørgensen et al., 1993) or sea bass (*Dicentrarchus labrax*) (Papoutsoglou et al., 1998). Stocking density also has an important role during the settling of larvae of Japanese flounder (*Paralichthys olivaceus*) (Bolasina et al., 2006), and it is also involved in fish welfare in many species (Ashley 2007), like rainbow trout (Ellis et al., 2002; North et al., 2006), Atlantic salmon (*Salmo salar*) (Turnbull et al., 2005) or Atlantic halibut (*Hippoglossus hippoglossus*) (Kristiansen et al., 2004). Senegalese sole shape can also be significantly affected by stocking density (Ambrosio et al., 2008).

Mechanisms relating stocking density and growth are not fully understood, but it is generally accepted that, when water quality is not affected by the increased number of fish per cubic meter, and food items are provided in sufficient amounts, differences in growth performance could be attributed to the onset of hierarchies and dominance relationships (Papoutsoglou et al., 1998, Bolasina et al., 2006). Moreover, intrinsic internal factors such as genotype or the interaction among genotype and ongrowing environment, could as well be related to growth performance (Bagley et al., 1994).

Growth is also modulated by sexual dimorphism in many species as sea bass (Gardeur et al., 2001a; Saillant et al., 2001) and turbot (Imsland et al., 1997), with females growing faster than males, although, up to our knowledge, no data are available about this particular issue for Senegalese sole.

In soleid fish contradictory results on how density affects growth have been given by different authors. Schram et al. (2006) found significant effects of density on growth in common sole, but Salas-Leiton et al. (2008) assaying four stocking densities between 2 and 30 kg m^{-2} with Senegalese sole did not find any significant differences in biomass production or growth rates.

Gardeur et al. (2001b) postulated that often growth experiments fail in finding significant differences between treatments due to the inter-individual variation, which diminishes the statistical power of many growth studies approached from the classical analysis of variance point of view. A way to overcome these problems is to work with individualized fish and to apply a proper statistical methodology to extract as much information from the data as possible. Mixed-effects models are a refinement of generalized linear models that take into account random effects as well as fixed effects to better describe the variance and covariance of the sample, thus providing a better resolution than generalized linear models. Fixed effects are unknown constants to be estimated from the data, while random effects influence the variance–covariance structure of the response variable. This method can be used when data present temporal pseudoreplication as each individual is measured several times as it grows during the course of an experiment (Crawley, 2007).

The aim of the present work was to take an individual-based approach to growth, and growth sexual dimorphism, of Senegalese sole reared at high and low stocking densities, by fitting linear mixed-effects models on individually tagged fish.

2. Material and methods

2.1. Density definition and experimental layout

Sole life habits are closely related to the sea bottom, including burrowing in sandy substrates to avoid predators or browsing for food items. Thus, a surface/surface criterion was chosen to define different densities over the mass/volume or mass/surface criterion, widely used in other fish species of symmetrical body. Density was thus calculated as a percentage of tank bottom covered by fish body surface. Following this criterion, 2 densities were defined: a low stocking density (LD) where enough bottom surface should make fish overlapping unnecessary (set at 60% of bottom surface covered by fish) and a high stocking density (HD) where fish overlapping was granted (set at 180% of bottom surface covered by fish). Fish surface estimation was individually calculated assuming that sole shape could be assimilated to an ellipse.

The three experimental tanks (0.88 m^2 of bottom surface and a volume of 700 l) were each equipped with two movable dividers that split each tank in 2 experimental units of independent and adjustable surface, and thus, allowing for the control of stocking density.

Fish were obtained from a fish farm in the Ebro river delta, in the NE coast of Spain, and conditioned to the experimental tanks at a low density (50% of bottom occupation) for 62 days at the Mediterranean Marine and Environmental Research Centre (CMIMA) in Barcelona, Spain. A total of 96 Senegalese sole (318.7 ± 7.9 g; mean ± standard error of the mean) were individually color-tagged (Reig et al., 2003), weighed, measured, and randomly distributed among the 6 experimental units (16 fish each). Subsequently, HD and LD treatments were randomly assigned to the 2 experimental units of each tank, and the available bottom surface for each treatment was set with the movable dividers. However, at day 77, due to fish size and tank size constraints, it was necessary to set a definitive value for available area and, thus, stocking density grew proportionally from then on. Stocking density at days 1, 134 and 195 are shown in Table 1.

The experiment lasted a total of 195 days and biometric data (weight (WG), standard length (SL), total length (TL) and maximum width (WD)) were gathered for each individual on days 1, 20, 40, 61, 77, 103, 126, 134, 147, 161 and 195. From days 134 to 195 treatments were reverted, in a way that fish under low density conditions were then under a high stocking density and vice-versa (albeit keeping their original names LD and HD), thus defining 2 experimental periods: period 1 (P1) from day 1 through day 134, and period 2 (P2) from day 134 through day 195.

On census days, all fish were anesthetized by immersion in sea water with MS-222 (200 mg l^{-1}), individually identified, measured and then digitally photographed, perpendicularly to their zenithal side, against a highly contrasted background provided with a printed scale.

To obtain a small sample of tissue to determine the RNA/DNA ratio throughout the experiment, a small biopsy of white epaxial muscle tissue was carried out with a 18 gauge cutting biopsy needle (Biopince, Amedic) at every census day except on days 1, 161 and 195 (Sánchez et al., 2003).

Besides the standardized biometric measures, centroid size (CS) for each individual was also computed from the digitalized images using the software tpsRegr v. 1.28 (Rohlf, 2003). Centroid size is a potentially interesting way of assessing fish growth, as it is a measure that is mathematically independent of shape in the absence of allometry (Zelditch et al., 2004).

At the end of the experiment, fish were sacrificed by anesthetic overdose and immersion in chilling water. Sex was determined by dissection and visual inspection of the gonad in all fish except 2 LD individuals.

2.2. Environmental conditions

Fish were kept in a flow-through circuit of sea water that flow into the tanks through a vertical pipe perforated every ten cm from the surface to the bottom of the tank in order to homogenize the environmental conditions as much as possible. Water flow (30% of the tank volume per hour), temperature (20 ± 1 °C), salinity (38.2 mg l^{-1}) and O_2 (>5.0 mg l^{-1}) were monitored daily. Photoperiod for 41.23° N latitude from July to January with artificial dusk and dawn was simulated with fluorescent light dimmed by shading covers laid over the tanks.

On day 77, a disease burst affected most of the individuals of one tank. This replicate was eliminated and, as a prophylactic measure, siliceous aquarium gravel (2 to 4 mm diameter) was added to the remaining tanks.