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EFFECTS OF PRE-WEANING FEEDING FREQUENCY ON GROWTH, SURVIVAL, AND DEFORMATION OF SENEGALESE SOLE, *SOLEA SENEGALENSIS* (KAUP, 1858)

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Abstract

Despite much interest in the production of Senegalese sole (*Solea senegalensis*) in southern Europe, weaning of this species onto artificial diets is problematic and varying results are obtained. The aim of this study was to test two feeding frequencies during a 13-day pre-weaning period and assess their impact on the growth and survival of Senegalese sole. Postlarvae were fed *Artemia metanauplii* with a peristaltic pump every hour for 12 hours per day or twice daily (morning and late afternoon). Both groups were suddenly weaned onto a commercial diet for an additional 30 days. At the end of the experiment, the relative growth rate and final dry weight were significantly higher and the survival significantly lower in the 12-hour treatment than in the twice-daily treatment. The feeding frequency had no effect on condition factor. The incidence of deformities was about 80% in both treatments.

Introduction

It is generally believed that a high feeding frequency maximizes growth in fish juveniles and larvae (e.g., Haylor, 1993), especially in pre-weaning stages when postlarvae are usually fed *Artemia metanauplii* exclusively (Houde, 1989; Conceição et al., 1997). Growth rates vary greatly and in many cases appear to be limited by food availability, as in Arctic charr (Miglavs and Jobling, 1989) and flounder (Lee et al., 2000). Animals compete

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intraspecifically for resources and it is generally assumed that more competitive individuals with a higher feeding rank have higher growth rates (Damsgård et al., 1997). Commercial hatcheries that produce marine fish generally supply food to postlarvae several times during the day or even continuously. This procedure may enhance the growth rate and decrease size variation, helping to shorten the time required to reach market size. More frequent meals reduce size dispersion in several species such as whitefish, *Coregonus lavaretus* (Koskela et al., 1997), and greenback flounder, *Rhombosolea tapirina* (Chen and Purser, 2001).

Skeletal abnormalities are a serious economic problem in aquaculture as they affect fish appearance and survival, reducing market value (Koumoundouros et al., 1997a). Nutritional factors have been indicated as possible causes for alterations in the normal development of skeletal structures that lead to abnormalities in adult fish (Takeuchi et al., 1995; Gapasin and Duray, 2001).

Studies of husbandry techniques in flatfish show that feeding amounts, frequencies, and durations significantly impact growth and homogeneity (Carter et al., 1996; Shelverton and Carter, 1998; Verbeeten et al., 1999; Chen and Purser, 2001). Senegalese sole *(Solea senegalensis)* postlarvae differ from other species not only because they settle on tank bottoms well before weaning but also because they display peculiar feeding behavior; settled Senegalese sole do not react readily to supplied food and prefer grazing on *Artemia* or dry diets from tank bottoms (Dinis et al., 2000).

Despite the high potential of Senegalese sole as an aquaculture species, only a few studies relate to larvae rearing conditions (Esteban et al., 1995; Dinis et al., 1999) and weaning periods (Marin-Magan et al., 1995; Cañavate and Fernández-Díaz, 1999). The aim of this study was to test two feeding frequencies with the same quantity of the same food during a 13-day pre-weaning period and assess the impact of feeding frequency on weaning success and postlarvae quality in Senegalese sole, *S. senegalensis*.

Materials and Methods

Fish and rearing. Senegalese sole larvae were reared until the beginning of the experimental period as described by Dinis et al. (1999). Newly hatched larvae were reared in a 200-l cylindro-conical tank in a closed recirculating system at a density of 100 larvae per liter. Larvae were fed rotifers *(Brachionus rotundiformis)* enriched with microalgae, *Isochrysis galbana* and *Tetraselmis chuii*, three days after hatching. At five days, *Artemia* sp. *nauplii* (Be 480 strain, INVE Aquaculture) were added to the diet. Rotifers were gradually reduced until day 8. After 10 days, *Artemia metanauplii* enriched with *I. galbana* and *T. chuii* were provided.

At 20 days, postlarvae were transferred to six 50-l white flat-bottom plastic tanks (surface area 0.5 m^2) in a closed recirculating system of 3000-l at 3000 individuals/m^2. *Artemia metanauplii* (RH strain, INVE Aquaculture) enriched with Super SELCO (INVE Aquaculture) were supplied to the postlarvae.

Environmental parameters were measured daily. Temperature and salinity averaged 20.9±0.6°C and 32.0±1.0‰, respectively. Dissolved oxygen was around 6.45±0.7 mg/l. A photoperiod of 12h light:12h dark was produced by overhead fluorescent tubes.

Tanks were cleaned and dead fish were removed and counted daily.

Food and feeding regime. The pre-weaning period started 26 days after hatching to avoid possible stress effects due to transfer from the cylindro-conical tanks to the flat-bottom tanks and ensure that fish were eating normally. Treatments were randomly assigned to the tanks, with three replicate tanks per treatment. In the pulse treatment, postlarvae were fed *Artemia metanauplii* by peristaltic pump every hour for twelve hours per day. In the second treatment, two meals were provided (morning and late afternoon). Both treatments received the same daily ration per fish. Between days 30 and 37, the *metanauplii* supply was gradually changed from live to frozen until, on days 37 and 38, postlarvae were fed frozen *metanauplii* exclusively. The postlarvae were weaned on day 40, after a one-day fast, and given AgloNorse no. 2 (0.6-1.0 mm) until day 63 and AgloNorse no. 3 (1.0-1.6 mm) afterward. The experiment ended on day 69. In both treatments, the inert diet was supplied by automatic feeders every hour for 18 hours a day. Throughout the experiment, it was attempted to feed close to satiation, based on the predicted maximum growth. Daily adjustments were based on visual inspection (to avoid excess uneaten food).

Sampling. At the end of the pre-weaning period, samples of twenty 40-day postlarvae were taken from each tank (60 postlarvae per treatment). At the end of the experiment, thirty 69-day postlarvae were sampled from each tank (90 postlarvae per treatment). The postlarvae were measured (total length) and kept frozen at -80ºC for dry weight determination. On day 69, samples taken for skeleton evaluation were fixed overnight in 4% formaldehyde buffered to pH 7.4 with PBS.

Fish were counted at the end of the weaning period to determine survival.

Skeleton evaluation. Specimens for skeleton evaluation were submitted to a double staining procedure using Alcian Blue 8GX to stain cartilage and Alizarin red S to stain bone, according to the procedure described in Gavaia et al. (2000). Specimens were preserved in glycerol until observation. Structural development was determined on the axial skeleton based on Gavaia et al. (2002) and eye migration was determined.

Data analysis. Relative growth rate (RGR, %/day) at the end of the experiment was calculated by the formula: $(e^{(g)}-1) \times 100$ where $g = (\ln_{final\ wt} - \ln_{initial\ wt})/(time)$ and e = Napier's constant (2.71). The coefficient of variation (CV) was determined as: (treatment standard deviation/treatment mean) x 100 to determine inter-individual weight and length variation among fish in the same treatment at the end of both periods. Food conversion ratio (FCR) was determined as: feed supplied to the tank/(final wt - initial wt) x number of fish per tank, where the proportion of food wasted (e.g., flushed out or dissolved) was considered negligible. The condition factor (K) was calculated as: (fish wt/total length^3) x 100. Data are presented as arithmetic means with standard deviations. One-way ANOVA was used to test differences between treatments. Differences were considered significant when $p<0.05$. When significant differences were found, Tukey's Honest Significant Difference (HSD) test was used to determine if the treatments differed significantly at $p<0.05$. All statistical analyses were carried out using the Statistica 5.1 and SigmaPlot packages software.

Results

Growth performance and survival. The mean dry weight at the end of the pre-weaning period (40 days) did not significantly differ between treatments and was 13.7±4.0 mg in pulse-fed fish and 13.9±3.0 mg in fish fed twice daily. At the end of the experiment (69 days), however, postlarvae from the pulse feeding regime were significantly larger (76.2±2.1 mg) than those fed twice daily (64.1±7.7 mg). Accordingly, the relative growth rate at 69 days was significantly higher in the pulse feeding treatment than in the twice-daily treatment (Table 1). The twice-daily feeding frequency significantly increased survival and the food conversion ratio at 69 days, while there was no significant difference between treatments in condition factor. The coefficient of variation of weight in the fish fed twice a day was significantly lower than for the pulse-fed fish at 40 days, but there was no significant difference at 69. These results are visible in the distribution of fish weights (Fig. 2). There was no significant difference in the coefficient of variation of length at either 40 or 69 days.

Skeletal evaluation. The most common deformities on caudal and pleural vertebra were the fusion and compression of vertebral *centra*, affecting adjacent neural arches and spines, however parapophysis was rare. The preural vertebrae 1-4 that contribute to the caudal fin internal skeleton were commonly fused or deformed, in some cases with an absence of neural or hemal arches. In the hypuralia, only hypurals 1-5 and the parhypural were affected. Abnormality in the hypuralia usually involved fusion of hypurals 1-2 or hypurals 3-5 and, sometimes, absence of any of these structures. The caudal, dorsal, and

Table 1. Survival, coefficient of variation (CV) for weight and length, condition factor (K), food conversion ratio (FCR), and relative growth rate (RGR) of Senegalese sole at weaning (40 days) and at the end of the experiment (69 days).

	Pulse feeding	*Fed twice daily*
40 days		
Survival (%)	100	100
CV (dry wt)	29.4±0.8[a]	21.8±3.2[b]
CV (length)	7.8±0.4	9.4±3.8
K	1.0±0.1	0.9±0.1
69 days		
Survival (%)	44.3±15.2[a]	69.8±14.8[b]
FCR	1.3±0.2[a]	1.7±0.2[b]
RGR (%)	6.3±0.7[a]	5.6±0.7[b]
CV (dry wt)	45.0±6.7	44.7±8.4
CV (length)	15.1±2.2	16.0±3.6
K	1.3±0.0	1.3±0.2

Values with different superscripts significantly differ ($p<0.05$).

anal fins were rarely affected, with only minor malformations of pterigophores and rays that were either shortened or abnormally bent. Cases of abnormal eye migration (uncompleted or no migration) were rare. There was no significant difference between treatments in number of skeletal abnormalities (Fig. 2), however the number of fish considered normal was low (~20%) in both treatments. In both treatments, some 60% of the fish had one or two deformities while fish with more than two deformities were less common.

Analysis of double stained specimens showed that the most affected structures were the caudal vertebra, adjacent arches, and spines. There were no significant differences in abnormalities between treatments, with the exception of the neural and hemal spines (Fig. 3). The number of deformed pleural vertebrae 1-4 was comparable to the neighboring area, presenting fusions between vertebral *centra* and malformations in the adjacent arches and spines.

Discussion

At the end of the pre-weaning period, the dry weight of the postlarvae was the same in both treatments and higher than values reported earlier (Cañavate and Fernández-Díaz, 1999). However, there was a significant difference in the coefficient of variation of the weight. The pulse-fed postlarvae had a greater weight distribution than those fed twice daily. A high coefficient of variation in fish may lead to aggressive behavior and/or reduced availability of food to less competitive animals (Jobling and Wandsvik, 1983). Despite the absence of apparent aggressive behavior in our study, the high coefficient of variation suggests that a feeding hierarchy

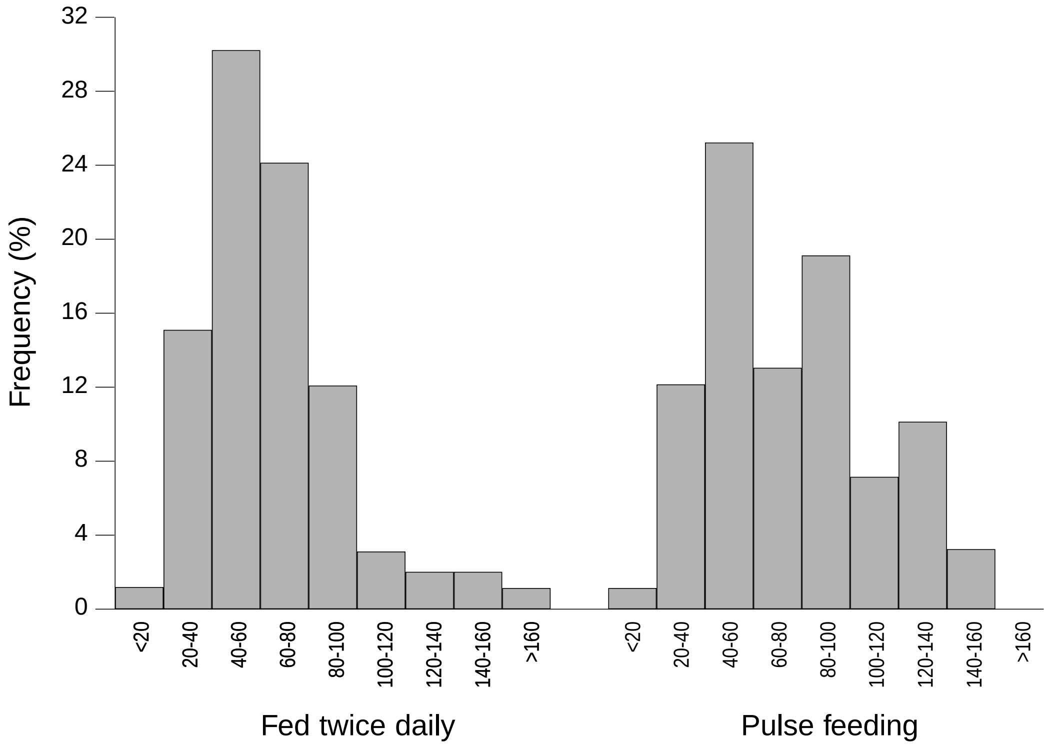

Fig. 1. Distribution of fish weight (n = 90) by treatment at the end of the experiment (69 days).

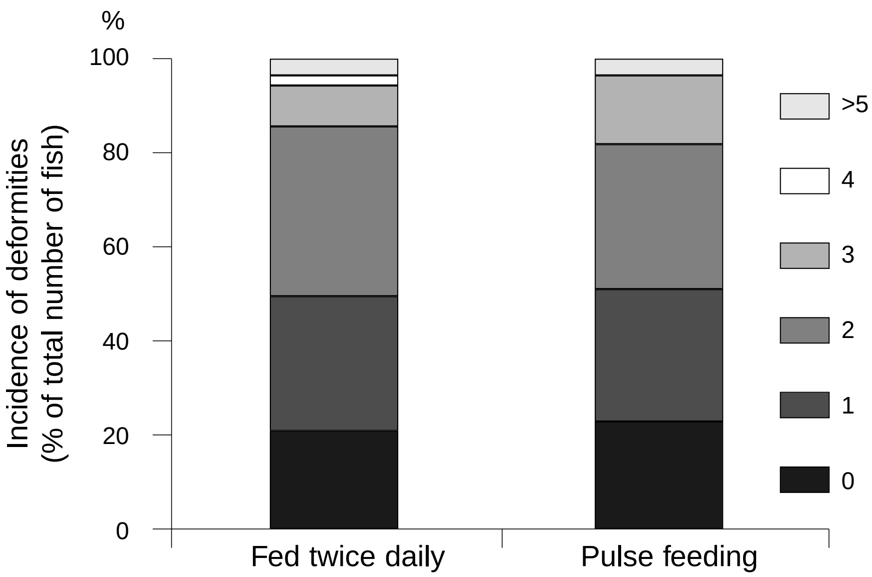

Fig. 2. Distribution of the number of deformities per fish at the end of the experiment (69 days).

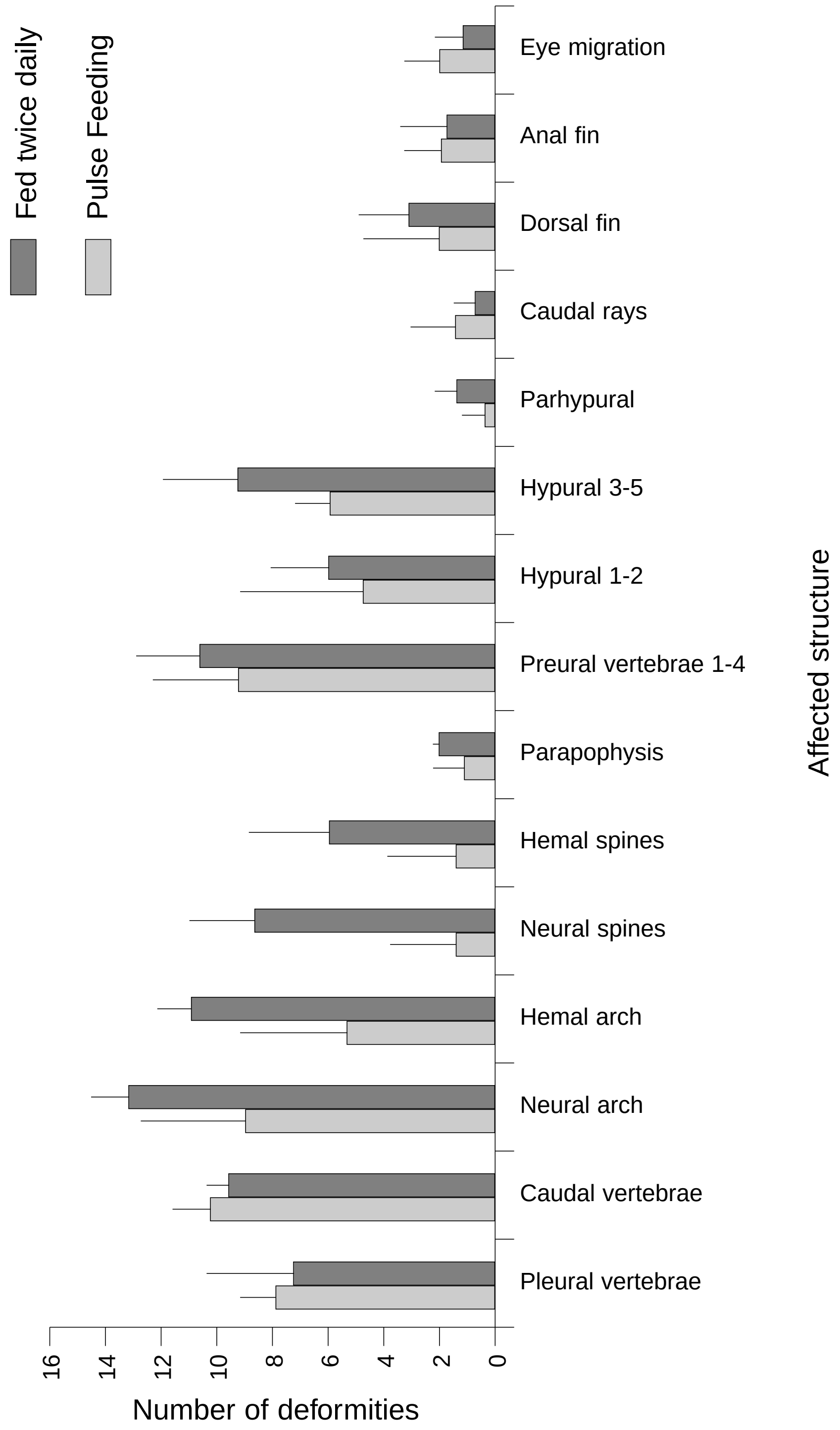

Fig. 3. Distribution of abnormalities by affected structure (n = 90) at the end of the experiment (69 days).

may have existed. It is unlikely that a lack of food availability caused the high coefficient of variation since the fish were fed in slight excess. The smallest fish in the pulse treatment were probably those that died during the weaning period, suggesting that they may have been under some sort of stress, nutritional or other, as suggested by Jobling (1982). Experiments with turbot indicate that larger juveniles cause stress to smaller fish, preventing them from obtaining a normal feed intake (Carter et al., 1996).

At the end of the experiment, survival rates were high compared to those in other studies (Cañavate and Fernández-Díaz, 1999). The high survival rates were possibly a result of different rearing and weaning techniques. At the end of our pre-weaning period, postlarvae were larger and suddenly weaned instead of fed both commercial feed and *A. metanauplii* as in Cañavate and Fernández-Díaz (1999). The pulse-fed sole had significantly lower survival than the twice-daily sole, but significantly better growth and food conversion. During feedings, Senegalese sole usually respond passively, similar to Arctic charr (Linnér and Brännäs, 2001). In these two species growth results were similar: fish fed more frequently grew better. Size-selective mortality in the pulse treatment may partly explain the absence of a difference in coefficient of variation for weight at the end of the experiment, in contrast to the end of the pre-weaning period.

The total number of individuals with skeletal abnormalities in both groups (~80%) was much higher than the 44% obtained for the same structures in postlarvae and juveniles in earlier studies (Gavaia et al., 2002). One explanation for the differences between studies may be different feeding regimes. In Gavaia et al. (2002), no rotifers were offered as first feeds and *A. metanauplii* were enriched only with microalgae. Similar figures and variability were obtained for *Paralichtys olivaceus* seedlings where 30-60% had malformations in the caudal complex (Hosoya and Kawamura, 1998). In *Sparus aurata*, the number of hatchery reared individuals with deformed caudal complex and vertebral column can reach 100% (Boglione et al., 2001). Although a high number of skeletal abnormalities were observed in our study, survival was comparable to previous studies of this species (own unpubl. results). Earlier studies suggest that malformations are induced in the early embryonic and larval stages, although the causes and mechanisms are not well understood (Koumoundouros et al., 1997b). Most axial skeleton structures appear, and probably acquire deformities, prior to and during metamorphosis (around 10-18 days after hatching). Therefore, differences due to dietary treatment would not be expected. The absence of significant differences between treatments in frequency of abnormalities indicates that skeletal abnormalities do not interact with pre-weaning feeding frequency to define selective mortality during the subsequent period.

In summary, the present study indicates that pre-weaning feeding frequency affects weaning performance in Senegalese sole. Pulse feeding produces fewer but larger fish while feeding twice daily leads to smaller fish with a higher survival rate. This suggests that mortality during weaning is selective (higher amongst smaller fish) under a pulse feeding regime but is unaffected by skeletal abnormalities.

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