

Research Articles

Stocking density and its influence on the productivity of red cusk eel, *Genypterus chilensis* (Guichenot, 1848), in shallow raceways

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ABSTRACT. We compared the growth properties of red cusk eel *Genypterus chilensis* with initial mean weight \pm standard error, 106.2 ± 6.1 g reared in shallow raceways at three different stocking densities (28.5, 46.1, and 60.7 kg m^{-3}) in a 226-day long growth trial at a constant temperature of 17°C . There was a trend towards higher specific growth rates at the highest density. Final mean weights were 333 ± 12 , 352 ± 12 , and 354 ± 15 g, at the 30, 45, and 60 kg m^{-3} density, respectively. Overall, the growth rates were higher in the 60 kg m^{-3} density group than the two other density groups. The daily feeding intake (%) was higher in the medium (0.51), and high (0.55) density groups compared the low-density group (0.45). Feed conversion efficiency (FCE) did not differ between the density groups. A significant size rank correlation was maintained in all density groups throughout the study. Calculated productivity increased almost linearly with increasing stocking density and was found to be 32, 34, and $39 \text{ g m}^{-2} \text{ d}^{-1}$ at 30, 45, and 60 kg m^{-3} , respectively. The results show that the optimum density conditions for farming juvenile red cusk eel, both concerning growth rate, feed conversion, and productivity is at densities equal to or higher than 60 kg m^{-3} .

Keywords: *Genypterus chilensis*; red cusk eel; stocking density; growth; shallow raceways; aquaculture

INTRODUCTION

The red cusk eel *Genypterus chilensis* (Guichenot, 1848) is a marine fish from the Ophidiidae family. Its geographical distribution extends from Paita in Peru to Cape Horn in southern Chile (Kong *et al.*, 1988; Vega *et al.*, 2018). It mainly inhabits coastal bodies of water at depths that generally range between 20 to 150 m; it is a benthic species of the continental shelf usually found on rocky substrates, feeding on fish, crustaceans, and mollusks (Chong *et al.*, 2006; Vega *et al.*, 2018). *G. chilensis* has been a highly attractive resource for the local market due to the excellent quality of the meat, which has led to the overharvesting of this species over the last 12 years, producing a sharp decline in landing volumes (Chong *et al.*, 2006; Vega *et al.*, 2018). One alternative to solve the unmet demand of *G. chilensis* is

its production through fish farming. Initial trials have focused on the development of incubation systems (Vega *et al.*, 2012), juvenile growth under culture conditions (Vega *et al.*, 2015), and reproduction of *G. chilensis* in captivity (Vega *et al.*, 2018). Initial growth trials (Vega *et al.*, 2015) in small quadrangular tanks (200 L) with three different size groups indicated that market size (2 kg) could be reached in 18-26 months from the initial size of 4 and 26 g, respectively. No published growth trials exist for the red cusk eel in on-growing systems.

The idea of using shallow raceway systems (SRS) for fish production appeared in the late eighties and has since been developed from an experimental scale into commercial large-scale production systems (*e.g.*, Øiestad, 1999; Labatut & Olivares, 2004; Imsland *et al.*, 2009a). The technology is especially well suited for

land-based production of flatfish like turbot, *Scophthalmus maximus* (Rafinesque); Dover sole, *Solea solea* (Linnaeus); Senegal sole, *S. senegalensis* (Kaup); and Atlantic halibut, *Hippoglossus hippoglossus* (Linnaeus), or bottom-dwelling species like the spotted wolffish, *Anarhichas minor* (Olafsen), as it opens possibilities for a reduced land requirement and building investments as well as reduced water consumption, by stacking the raceways in several levels and re-using the water from level to level. The compactness and the system's self-cleaning properties, combined with high stocking density, are important characteristics of the shallow raceway system. Although the high density is considered a crucial property of the shallow raceway system, very little information exists on the effect of high density on the growth of candidate species reared in such a system.

Stocking density is a major factor affecting fish growth under farmed conditions (Kjartansson *et al.*, 1988; Tremblay-Bourgeois *et al.*, 2010; Calabrese *et al.*, 2017). Both negative and positive relationships between stocking density and growth have been reported, and the pattern of this interaction appears to be species-specific (Jørgensen *et al.*, 1993; Björnsson, 1994; Irwin *et al.*, 1999; Imsland *et al.*, 2009a). However, no density studies have been performed and published for red cusk eel, and neither has the culture of the species in an SRS system been tried. Therefore, the following experiment was designed to investigate the effects of different rearing densities on the growth rate, size hierarchies, daily feed intake, feed efficiency ratio, and red cusk eel productivity in a shallow raceways system.

MATERIALS AND METHODS

Fish stock and rearing conditions

This experiment was undertaken between January and October 2018 at Colorado Chile S.A. facilities located in Panul Bay (33°18'S, 37°18'W), Coquimbo, northern Chile. This study was conducted in a land-based recirculation aquaculture system facility designed for the red cusk eel *Genypterus chilensis* pre-rearing aquaculture stage. Red cusk eel juveniles were reared in shallow raceways and fed using a species-specific diet formulated by Cargill until the experiment. In December 2017, the fish were distributed randomly into six raceways (0.45×0.90 m, 0.40 m²) with a water level of 26 cm, each providing a total volume of 0.10 m³. The water flow was set to 20 L min⁻¹ for each of the experimental units. In the shallow raceways, the water inlet pipe was in front of perforated screens. The inlet flow was pointed against the wall and away from the screens to avoid jet currents. Oxygen saturation was

measured with a hand-held oxygen meter (OxyGuard Handy Polaris, OxyGuard International A/S, Denmark) at regular intervals in all raceways' effluent water. Adult red cusk eel prefers low light rearing conditions, and based on the experience gained by the fish farmer; it gets easily startled if much light is used. Accordingly, the fish were kept in the dark apart from during the period of daily feeding and cleaning routines.

Experimental design

The growth study was carried out from January 24, 2018 until October 17, 2018 with 255 red cusk eels. In each raceway, 20 fish were individually tagged using Trovan[®] Passive Transponder tags. Water was supplied from a recirculating aquaculture system (RAS) at a constant temperature of 17°C, and oxygen levels were kept above 70% in the water outlet. Three experimental densities were applied: 28.5 (low), 46.1 (medium), and 60.7 (high) kg m⁻³ with two replicate raceways for each experimental group. Hence, the experimental set-up is a two-way nested design, where the two replicates are nested within the experimental groups.

There are no published density studies on red cusk eel, but the North-Atlantic species spotted wolffish display many similar characteristics, including a similar body shape. In a density trial with spotted wolffish, Jonassen (2002) tested density in the range 20-60 kg m⁻² for large juveniles and as no published trials existed on red cusk eel density, we used a similar range in this experiment.

All fish were anesthetized (benzocaine, 0.05 g L⁻¹) and measured approximately monthly intervals throughout the experiment. Growth results are based on the tagged fish's registration, and other results are based on total biomass registrations.

The fish were fed twice a day using a species-specific diet formulated by Cargill. The first feeding was around 09:00 h, and the second feeding around 14:00 h. fish were fed until uneaten feed pellets were observed on the tank bottom when the feeding was halted, to ensure satiation. Exact feeding registration was done in the first 60 days of the experiment, *i.e.*, one for the first growth period (January-February) and the second one from February to March. There are two replicates for each experimental group and two measurement groups, *i.e.*, n = 4, for each value in Table 1.

Feed data

Total feed consumption (C_T) was defined as the total feed supplied in each rearing unit daily. Daily feeding rate (F%) was calculated as

$$F\% = 100 [C / ((B_1 + B_2)/2)] (t_2 - t_1)^{-1}$$

Table 1. Average daily feeding rate (F) and feed conversion efficiency (FCE) of red cusk eel *Genypterus chilensis* reared at different densities in shallow raceways. Results are given as arithmetic mean \pm standard deviation for the two experimental periods where feeding data was sampled (*i.e.*, $n = 4$ for each experimental group). Different superscript letters denote significant differences between the experimental groups (Student-Newman-Keuls multiple comparisons, $P < 0.05$).

Experimental group	F%	FCE
Low density	0.45 ± 0.04^b	1.63 ± 0.50
Medium density	0.51 ± 0.03^a	1.37 ± 0.31
High density	0.55 ± 0.02^a	1.38 ± 0.59

where C is feed consumption (g dry matter) in the period, and B_1 and B_2 are fish biomass (g wet weight) on days t_1 (start) and t_2 (final), respectively. Feed conversion efficiency (FCE) was calculated as biomass gain per weight unit of consumed feed:

$$FCE = (B_2 - B_1) / C$$

Data analysis and statistical methods

Specific growth rate (SGR) was calculated according to the formula:

$$SGR = (e^g - 1) \times 100$$

where $g = (\ln W_2 - \ln W_1) (t_2 - t_1)^{-1}$ and W_2 and W_1 are wet weights (g) at days t_2 and t_1 , respectively.

Productivity expressed as the amount of red cusk eel produced per m^2 was calculated using the formula of Schram *et al.* (2006):

$$P = \frac{(W_f - W_0)}{A \times T}$$

where P: productivity (g $m^{-2} d^{-1}$); W_f : final biomass; W_0 : initial biomass; A: mean rearing area (m^2); T: number of days.

A two-way nested ANOVA (Searle *et al.*, 1992), where the two replicates were nested within density treatment, was applied to calculate the effect of different rearing density on mean weights and specific growth rates. The model equation of the nested ANOVA had the form:

$$X_{ijk} = \mu + \alpha_i + B_{ij} + \varepsilon_{ijk}$$

where μ is the general level; α_i is the treatment effect (*i.e.*, density); B_{ij} is the contribution caused by replicate (here: raceways a and b) j in group i and ε_{ijk} the error term. Significant ANOVAs were followed by a Student-Newman-Keuls multiple comparison test (Zar, 1984) to locate differences among treatments. For parameters where only group data existed (FCE, and F%), a two-way nested ANOVA was applied, followed

by a Student-Newman-Keuls multiple comparison tests to locate any differences among treatments.

Individual growth trajectories (IGT) were analyzed using a growth curve analysis (GCM) multivariate analysis of variance (MANOVA) model (Timm, 1980; Chambers & Miller, 1995). The model equation of the GCM had the form:

$$Y_{(n \times p)} = X_{(n \times q)} B_{(q \times p)} + E_{(n \times p)}$$

where $Y_{(n \times p)}$ are the growth vectors

$$y = (y_1, y_2, \dots, y_p)$$

for each p separate measurements on n individual fish; $X_{(n \times q)}$ is the design matrix or the set of variables measured for each individual, *i.e.*, $q = \text{age}_p + \text{density}_j + \text{replicate}_j$; ($i = 30, 45$ or 60 kg m^{-3}), ($j = \text{replicate a, replicate b}$); $B_{(q \times p)}$ is the matrix of parameters estimated by the model; $E_{(n \times p)}$ is the matrix of deviations for each individual from the expected value of $Y = XB$.

Size ranking (initial size rank *vs.* final size rank) and growth rate ranking (initial SGR *vs.* final SGR and SGR's in adjacent periods) was tested using Spearman's rank correlation (r_{sp}) (Zar, 1984). A Chi-squared test (Zar, 1984) was used for possible differences in the mortality of the tagged fish.

All statistical analyses were conducted using Statistica™ 13.3 software. A significance level (α) of 0.05 was used in all statistical tests if not stated otherwise.

RESULTS

Mortality

During the experiment, five fish (4.2%) died in the three density groups (1, 2, and 2 tagged fish in low-, medium- and high-density groups, respectively), and the mortality did not vary between the experimental groups ($\chi^2 < 2.6, P > 0.10$).

Growth: effect of density

The initial mean weight \pm standard deviation (SD) was 106.1 ± 3.7 g and did not differ significantly between the three densities (two-way nested ANOVA, $F_{2, 112} = 4.5, P > 0.15$, Power $(1 - \beta) > 0.3$, Fig. 1). Final mean weights were 354.2 ± 15.2 , 352.1 ± 12.5 and 333.1 ± 12.6 g for the high, medium and low-density groups, respectively. Specific growth rate (SGR) varied between the three density groups (two-way nested ANOVA, $P < 0.05$, Fig. 2), and the high-density group had the highest growth initially and during March-April (SGR4) and July-August (SGR8). Overall growth was also highest in the high-density group (SNK *post-hoc* test, $P < 0.05$).

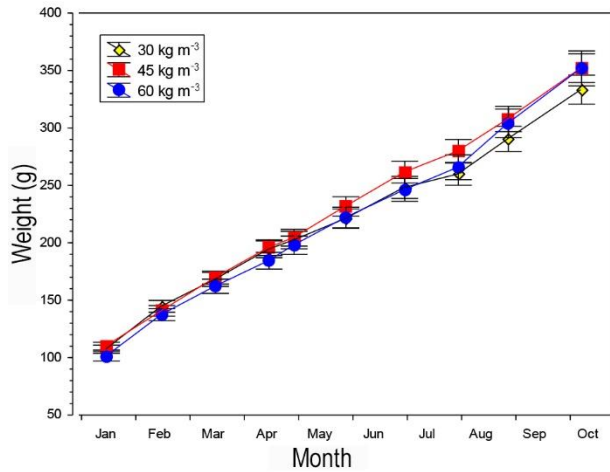


Figure 1. The mean weight of red cusk eel *Genypterus chilensis* reared in at different densities in shallow raceways. The vertical line indicating standard error may be obscured by symbol. The values for the two replicates are combined. $n = 37-40$ for each mean value. Diamond: 30 kg m^{-3} , square: 45 kg m^{-3} , circle: 60 kg m^{-3} .

Overall, the mean individual growth trajectories (IGT) differed between the density groups, indicating that longitudinal growth profiles of the density group differ (GCM, $\text{MANOVA}_{\text{Density}}$, Wilk's lambda (Λ)_{18, 210} = 0.36, $P < 0.01$), and the IGT were non-parallel between the groups, indicating differences in the groups IGT over time (GCM, $\text{MANOVA}_{\text{Density} \times \text{Time}}$, Wilk's lambda (Λ)_{16, 212} = 0.38, $P < 0.01$).

Feed Intake and feed conversion efficiency

Average feed conversion efficiency did not differ among density groups (Table 1). However, average feed intake differed between the density groups and was higher in the 45 (medium) (0.51) and 60 (high) (0.55) kg m^{-3} groups (two-way nested ANOVA, $F_{2, 6} = 8.5$, $P < 0.05$, Table 1), compared to the 30 kg m^{-3} (low) density group (0.45).

Size and growth ranking

A significant size rank correlation (initial weight vs. final weight) indicating stable size hierarchies was in all density groups ($r_{Sp} > 0.69$, $P < 0.05$), and was highest in the high-density group ($r_{Sp} = 0.79$). Positive correlations between initial and final growth rates were not found in any density group ($r_{Sp} < 0.19$, $P > 0.20$). However, correlations between growth rates in adjacent periods (*i.e.*, period_n and period_{n+i}) were only found once in the medium-density groups ($r_{Sp} = 0.32$, $P < 0.05$, Table 2) and not seen in the high-density group, whereas this was seen in four out of eight growth periods in the low-density group ($r_{Sp} > 0.32$, $P < 0.05$, Table 2).

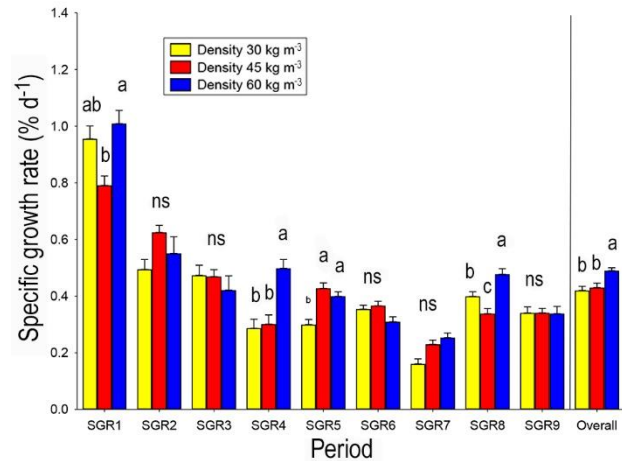


Figure 2. Specific growth rates of red cusk eel *Genypterus chilensis* reared in at different densities in shallow raceways. The vertical line indicates standard error. Different letters indicate statistical differences (two-way nested ANOVA, $P < 0.05$), with 'a' as the highest value, 'b' as the second highest, and 'c' as the lowest value, ns not significant. The values for the two replicates are combined. $n = 37-40$ for each mean value.

Productivity

The relation between productivity as achieved in this experiment and stocking density is shown (Fig. 3). Productivity increased almost linearly with increasing stocking density and was calculated as 120, 129, and $146 \text{ g m}^{-2} \text{ d}^{-1}$ at low, medium, and high density respectively.

DISCUSSION

Stocking density is a crucial element in the rearing of demersal species like red cusk eel *Genypterus chilensis* and, there is a lack of systematic investigation on the subject for this species as previous studies have focused on the development of incubation systems (Vega *et al.*, 2012) and reproduction of red cusk eel in captivity (Vega *et al.*, 2018). The only previously published growth trial (Vega *et al.*, 2015) on red cusk eel investigated growth in small quadrangular tanks (200 L) with three different size groups indicated that market size (2 kg) could be reached in 18-26 months from the initial size of 4 and 26 g, respectively. Information about density-dependent growth in other demersal species is available, albeit not conclusive. In general, the available data on demersal fish are data from short term density trials with juveniles (turbot, *Scophthalmus maximus*, Irwin *et al.* (1999); winter flounder, *Pseudopleuronectes americanus*, Fairchild & Howell (2001); spotted wolffish, *Anarhichas minor*, Jonassen (2002); Dover sole, *Solea solea*, Schram *et al.* (2006); California halibut, *Paralichthys californicus*, Merino *et al.* (2007), whereas data for larger fish are scarce (however

Table 2. Correlations between specific growth rates (SGR) in adjacent periods (*i.e.*, period_n and period_{n+1}) of red cusk eel *Genypterus chilensis* reared at different densities in shallow raceways. Results are shown as Spearman's rank correlation (r_{Sp}) for each adjacent period along, number (n) of valid measurements in each group, and *P*-value. *Significant values ($P < 0.05$).

Pair of variables	Experimental group	n	Spearman's rank correlation	<i>P</i> -value
SGR1 & SGR2	Low density	39	-0.084	0.611
SGR2 & SGR3	Low density	39	0.412	< 0.010*
SGR3 & SGR4	Low density	39	0.412	< 0.010*
SGR4 & SGR5	Low density	39	0.601	< 0.001*
SGR5 & SGR6	Low density	39	0.250	0.123
SGR6 & SGR7	Low density	39	0.251	0.121
SGR7 & SGR8	Low density	39	-0.069	0.674
SGR8 & SGR9	Low density	38	0.326	< 0.050*
SGR1 & SGR2	Med. density	40	0.319	< 0.050*
SGR2 & SGR3	Med. density	40	0.253	0.114
SGR3 & SGR4	Med. density	40	0.071	0.661
SGR4 & SGR5	Med. density	40	0.016	0.918
SGR5 & SGR6	Med. density	40	0.231	0.151
SGR6 & SGR7	Med. density	40	0.113	0.484
SGR7 & SGR8	Med. density	40	0.228	0.156
SGR8 & SGR9	Med. density	38	0.307	0.056
SGR1 & SGR2	High density	39	0.092	0.573
SGR2 & SGR3	High density	39	0.114	0.486
SGR3 & SGR4	High density	39	0.231	0.260
SGR4 & SGR5	High density	39	-0.089	0.589
SGR5 & SGR6	High density	39	0.171	0.197
SGR6 & SGR7	High density	39	0.207	0.205
SGR7 & SGR8	High density	39	0.198	0.225
SGR8 & SGR9	High density	37	0.302	0.065

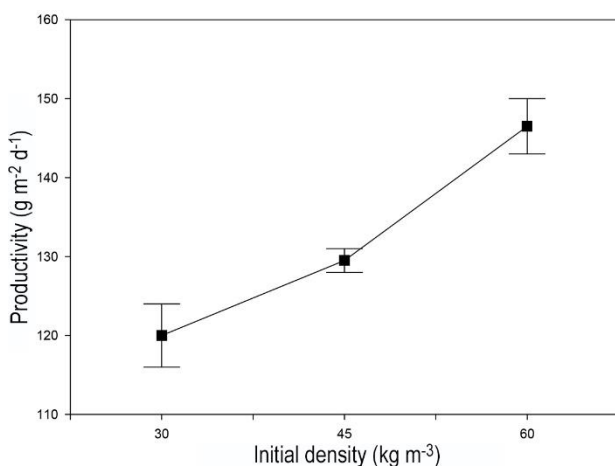


Figure 3. The relation between stocking density and productivity in red cusk eel *Genypterus chilensis*.

see data on large spotted wolffish (Imstrand *et al.*, 2009a). Irwin *et al.* (1999) reported the best growth for turbot juveniles (between 8-40 g) at medium (6 kg m⁻²) and high (7 kg m⁻²) densities. No effect of stocking density on growth was found for juvenile (0.2-2 g)

winter flounder up to 350% (3 kg m⁻²) bottom coverage (Fairchild & Howell, 2001). Schram *et al.* (2006) reared juvenile (20-50 g) Dover sole at densities between 0.6 and 12.6 kg m⁻² and found that Dover's growth rate significantly decreased with increasing stocking density.

For larger fish, Björnsson (1994) reported the optimal density of Atlantic halibut *Hippoglossus hippoglossus* to be between 20-40 kg m⁻² for 1 kg fish and between 25-55 kg m⁻² for 2 kg fish. Martínez-Tapia & Fernández-Pato (1991) reared turbot (6-1000 g) at two densities and reported higher growth for turbot reared at a final density of 68 kg m⁻² (initial density of 0.5 kg m⁻²) compared to a final density of 17 kg m⁻² (initial density of 0.25 kg m⁻²). Imstrand *et al.* (2009a) studied the growth of spotted wolffish with initial mean weight 3.65 ± 0.15 kg reared in shallow raceways at three different densities (50, 70, and 90 kg m⁻², corresponding to approx. 170, 240 and 310 kg m⁻³, respectively) in a 447-day long growth trial. They found that there was a trend towards higher specific growth rates at the highest density. All these studies indicate that growth may be equally good or better at

medium and high densities than low densities, which is in line with current findings on juvenile red cusk eel.

There are no published density studies on red cusk eel. However, the north Atlantic species spotted wolffish display many similar characteristics, including similar body shape and low light preference. Some published density trials from that species can give some insight into the effect of different density on growth in these bottom-dwelling species (*e.g.*, spotted wolffish, Jonassen, 2002; Imsland *et al.*, 2009a ; Tremblay-Bourgeois *et al.*, 2010). Jonassen (2002) and Tremblay-Bourgeois *et al.* (2010) both found a negative effect of high density on growth, whereas we found the opposite result in the present study. A reduced feed intake explained the negative impact of stocking density found by Jonassen (2002) and Tremblay-Bourgeois *et al.* (2010) in the high-density group, which might cause increased social interactions between individuals (Irwin *et al.*, 1999; Lambert & Dutil, 2001). In the study of Imsland *et al.* (2009a), feed intake was not reduced in the high-density groups. However, there was a tendency towards higher feed conversion efficiency at higher densities indicating that social interactions did not cause a higher degree of competition at high densities, similar to present findings where both growth and feed intake increased with higher density. Hence, the positive impact of high density on growth in red cusk eel seen in the present study may be at least partly explained by the increased feeding intake seen for this group. The present study was not exactly designed to examine feeding; this was extra information provided by the fish farmer (Colorado Chile S.A.). Future studies using similar, or higher, densities should also consider behavioral components that allow a better understanding of social interaction (*e.g.*, size hierarchies), growth, and feeding.

Density is tightly linked with the formation of size hierarchies and social interactions (Imsland *et al.*, 1998, 2009b; Kooijman, 2009). To enlighten whether and to which extent social interactions in turbot contribute to observed size variation in large rearing groups, Imsland *et al.* (1998) used a growth model simulation study and the size variation of the model data compared with the laboratory data of Sunde *et al.* (1998). Although difficult to measure directly in the form of size hierarchies, the results showed that social interactions do contribute to size variation in demersal fish culture (Imsland *et al.*, 1998). Some studies indicate that intraspecific competition and agonistic interaction is, in fact, greater when fish of similar size are reared together (Doyle & Talbot, 1986; Baardvik & Jobling, 1990; Imsland *et al.*, 1998). Imsland *et al.* (2009a,b) pointed out that this has two important consequences:

- a) a certain level of size variation is needed to reduce the adverse effects of social interactions, and
- b) demersal fish must be reared at optimal density as too low density will reduce growth due to a higher social interaction level.

Correlation between adjacent growth rates was seen in this study but was much more pronounced in the low-density group. It could indicate that stringent social hierarchies may have formed in the low-density group, which may have negatively affected growth as growth can be suppressed by competition under such conditions (Imsland *et al.*, 1998). Further, we found high size rank correlation observed at in all density groups in the present study that may indicate the establishment of stable size ranks (hierarchies), which is common under culture conditions (Huntingford *et al.*, 1990; Imsland *et al.*, 1998). It has been pointed out by Klokseth & Øiestad (1999) and Øiestad (1999) that less antagonistic behavior can be expected in the shallow raceway system compared to other rearing systems. As feed is floating and passing all individuals in the system, there will be less competition. Such a mechanism was indicated by Imsland *et al.* (2007) as less stringent formations of hierarchies were found in shallow raceways than conventional tanks.

In general, only a minor difference in mean weights was seen in the present study. However, the present findings' actual value is presented in the productivity calculations, as there is an almost linear increment in productivity between low, medium, and high density (Fig. 3). It is necessary to operate with the highest possible stocking densities in each rearing unit without compromising the fish's water quality or welfare to maximize productivity. As with any farming system, the shallow raceways system (SRS) success depends on good water quality control facilitated by the one-directional current (plug-flow) and the homogeneity of water quality at an SRS outlet. The high degree of water-reuse to be applied must be coupled with substantial monitoring and control of waste products. The volume of water that has to be monitored and treated is considerably less than conventional technology (about 20%). Thus, easier to deal with previous trials with demersal fish species in SRS have not found any negative effect on growth, blood physiology, or water quality (Labatut & Olivares, 2004; Imsland *et al.*, 2007, 2009a), and the system is cost-effective for production of turbot (Bjørndal & Øiestad, 2010) and sole (Bjørndal *et al.*, 2016). This maximum stocking density should not have negative consequences on growth (Björnsson, 1994; Lambert & Dutil, 2001; Schram *et al.*, 2006; Imsland *et al.*, 2009a; present study) or the general welfare of the fish (*e.g.*,

disease susceptibility, Fairchild & Howell, 2001; induction of stress response, Ruane *et al.*, 2002; present study Xavier Gutiérrez, NIVA Chile, *pers. comm.*). As no such negative effects were apparent in the present study, it may be deduced that maximum productivity of juvenile red cusk eel is reached at densities similar to the high-density group initial density of 60 kg m⁻³ in the present study.

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