
The effect of density on sea bass (*Dicentrarchus labrax*) performance in a tank-based recirculating system

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Abstract:

Sea bass (*Dicentrarchus labrax*) (135 ± 4 g) were reared under tank-based recirculating aquaculture system for a 63-day period at four densities: 10, 40, 70, 100 kg m⁻³. Fish performance, stress indicators (plasma cortisol, proteonemia plus other blood parameters—Na⁺, K⁺, glucose, pH, total CO₂) and water quality were monitored. At the end of the 63-day period, resistance to infection was also studied by a nodavirus challenge. A 25-day test was performed on fish from two extreme densities (10 and 100 kg m³) and one intermediate density (40 kg m³).

With regards to the different density treatments, there was no significant difference between the daily feed intake (DFI) and the specific growth rate (SGR) up to a density of 70 kg m⁻³. No significant difference was found between treatments concerning the feed conversion ratio (FCR) and the mortality rate. No density effect was observed on the fish stress level (plasma cortisol) or on sensitivity to the nodavirus challenge. Under these experimental rearing conditions, the density above 70 kg m⁻³ has an impact on growth performance (DFI and SGR) indicators and also some blood parameters (CO₂) at the highest density tested (100 kg m⁻³).

This study suggests that a density up to 70 kg m⁻³ has no influence on sea bass performance and welfare. At 100 kg m⁻³, average specific growth rate was decreased by 14% without welfare deterioration according to the welfare indicators monitored.

Keywords: Density; Recirculating system; European sea bass; Performance; Welfare

1. Introduction

Today, consumer demand for safe and ethically defensible food products is very much on the increase. Production systems must consider animal well-being and good health. Those factors depend on proper care and good husbandry practices. With regards to aquaculture, recirculating production systems are often associated to high density in tanks. Previous studies analysed the effect of increasing density on biological performance in fish, with positive and negative impacts observed according to the species (Irwin *et al.*, 1999). Generally speaking, high density is considered as a potential source of stress, with a negative effect on fish growth rate (Lefrançois *et al.*, 2001) and survival and feeding rates (Rowland *et al.*, 2006). Daily feed intake and specific growth rate decreases were observed at increasing density levels on several species such as Atlantic cod (*Gadus morhua* L.) (Lambert and Dutil, 2001), brook charr (*Salvelinus fontinalis*) (Vijayan and Leatherland, 1988), gilthead sea-bream (*Sparus aurata*) (Canario *et al.*, 1998) and largemouth bass (*Micropterus salmoides*) (Petit *et al.*, 2001). Negative effects of high density on fish performance are generally associated to water quality deterioration or/and increase of aggressive behaviour (Ellis *et al.*, 2002).

Most of the studies on the impact of density on fish performance have been carried out with freshwater species, mainly salmonids. Little information is available for marine species (Turnbull *et al.*, 2005). Studies were carried out on Atlantic halibut (*Hippoglossus hippoglossus*) (Kristiansen *et al.*, 2004), Dover sole (*Solea solea*) (Schram *et al.*, 2005), Atlantic salmon (*Salmo salar*) (Irwin *et al.*, 1999), gilthead sea-bream (*Sparus aurata*) (Canario *et al.*, 1998) but few on European sea bass (*Dicentrarchus labrax*) (Roncarati *et al.*, 2006). Sea bass is one of the leading Mediterranean cultivated species (Papoutsoglou *et al.*, 1998) and is classically produced in cages. In view of difficult accessibility of coastal areas due to competition with other activities, recirculating aquaculture systems (RAS), which require less space, represent an important alternative to marine fish production in cages. In the present context of water resource deterioration, RAS also present an opportunity to reduce water consumption and effluent emission by a factor of 100 in comparison to classical flow through systems (Blancheton, 2000) and allow concomitant control of rearing water quality. But little information is available on the effect of density on fish performance under intensive RAS conditions (Chandoo *et al.*, 2004). In sea bass, no differences in growth, survival, plasma cortisol glucose, total proteins, triglycerides and cholesterol were observed in fish reared in recirculating tanks at different densities up to 45 kg m⁻³ (Di Marco *et al.*, 2008).

Classical performance parameters, such as growth and survival rates (Jørgensen *et al.*, 1993; Canario *et al.*, 1998; Papoutsoglou *et al.*, 1998; Irwin *et al.*, 1999; Sørum and Damsgård, 2004) and blood parameters, such as plasma cortisol and glucose level (Kjartansson *et al.*, 1988; Mazur and Iwama, 1993; Montero *et al.*, 1999), can be used to measure the potential effect of density on fish performance and welfare. An alteration of serum protein concentration (proteinemia) has been also reported in relation to stress conditions in some species (Jeney *et al.*, 1997; Coeurdacier and Dutto, 1999; Magnadóttir *et al.*, 1999).

The aim of the present study was to assess the effect of density on sea bass performance, stress and resistance to infection in a recirculating system. This study was part of the Wealth Project (Welfare and health in sustainable aquaculture), that associated European researchers and farmers from 2004 to 2007, to define objective health and well-being criteria for aquaculture. Large range of densities are commonly applied in European farms, from 10 to more than 100 kg m⁻³, according to the rearing system, the fish species and size (Ellis *et al.*, 2002). During the 88 day experiment, fish performance, stress and resistance to infection were monitored at four different stabilized densities: 10, 40, 70 or 100 kg m⁻³.

2. Materials and methods

2.1. Fish management and feeding

2.1.1. The acclimatization phase

Sea bass were supplied by Méditerranée Pisciculture (Salses-le-Château, France), a partner farm for the Wealth project. Fish were stored for 2 months at 70 kg m⁻³ in a circular tank. During this phase of acclimatization to the RAS conditions and the tank design, fish were fed at 1.5% of biomass per day. They were also treated with Tugon 80 against a branchial parasite *Diplectanum aequans* (dose of 0.5 g m⁻³ during 48 hours).

2.1.2. The 63 day experiment

Fish (mean body weight 135 ± 4g S.D.) were starved for 24h, anaesthetized with 2-phenoxyethanol and then sorted for allocation to experimental tanks randomly. During 63 days, the fish were held in separate tanks at four different densities: 10, 40, 70 and 100 kg m⁻³. The experiment was divided into 3 periods of 21 days each (called P1, P2 and P3) to adjust tank densities at the end of each period by biomass random removal. Fish were manually fed, *ad libitum*, once a day (in the morning). Three criteria were used to assess satiation: (1) indifference of the fish for pellets, (2) first uneaten pellets on the tank bottom and (3) first pellets collected in the particle trap of each tank. Uneaten pellets collected in the particle traps were counted after each meal. The extruded commercial feed (Neo Grower Extra Marin, Le Gouessant) was composed of 45% of proteins and 20% of lipids, and presented a digestible energy of 18.5 MJ per kg of feed⁻¹.

2.1.3. The Nodavirus challenge

At the end of the 63 day experiment, a challenge test was performed during 25 days on the fish from 3 different densities: two extreme densities (10 and 100 kg m⁻³) and an intermediate density (40 kg m⁻³), corresponding to an usual density in commercial systems.

Fish were inoculated (intramuscular inoculation) with 0.2 ml of live Nodavirus suspension SW80 (9*10⁵ PFU per fish) as described by Coeurdacier *et al.* (2003). Thirty fish from each density treatment were placed in triplicate tanks (1m³). The tanks were connected to the same RAS and water temperature was maintained at 25°C. As a control, 30 fish from each density treatment were inoculated with sterile 0.5% NaCl solution. The tank used for the control group was connected to a separate RAS to avoid Nodavirus contamination. Total morbidity (i.e. mortalities and fish showing abnormal swimming behaviour) was recorded daily.

2.2. Experimental design

The experimental facility was composed of 12 circular tanks (1 m³ each) all working in RAS. The fish population was divided on the basis of four density treatments, 10, 40, 70 and 100 kg m⁻³ with each density treatment randomly allocated to triplicate tanks.

The experimental RAS was fed with pumped sea water and three water loops were used in order to optimise water management. The first loop generated water circulation with a pump system, the second loop produced super-oxygenated water (at 25 mg l⁻¹) due to a cone system and the third was the treatment loop described by Blancheton (2000). Thanks to the treatment loop, the same water quality was maintained in tanks independently of the density. This treatment loop including the CO₂ degassing column, mechanical, biological and UV filters, was designed to keep the rearing water quality below the recommended values for aquaculture (Pierce *et al.*, 1993; Person Le Ruyet *et al.*, 1997; Colt, 2006).

The pH was controlled using soda addition to keep values around 7.0-7.4 (Fivelstad *et al.*, 1995; 1998). Water temperature was maintained at the optimal level for growth at around 24°C (Person-Le Ruyet *et al.*, 2004) using a heat exchanger. Make up water was filtered twice (40 µm and 15 µm) before use. Tank water flows were measured and adjusted before the experiment in order to be proportional to the fish density per tank (Table 1). It was necessary to double the water flow rate per kg of biomass in the triplicate 10 kg m⁻³ tank to ensure sufficient water circulation and avoid faeces settling in the tanks.

2.3. Measured parameters

2.3.1. Water quality parameters

Temperature and salinity were measured daily in all tanks, with a Checktemp1 pocket Thermometer (Hanna instruments) and an ATAGO hand refractometer S/Mill-E. The pH and oxygen were also measured every day in all tanks using an Ecoscan® pH meter and a YSI 550A oxymeter. Oxygen concentration was monitored using Oxyguard® probes connected to a computerized measuring system (Linde soft). Carbon dioxide concentration was measured in samples once a week using a calibrated non-dispersive infrared (NDIR) gas analyzer (Li-cor CO₂ analyser).

Water samples were taken during the post-prandial period (when the excretion by the fish was maximal) and stored after filtration until analysis in appropriate conditions (preserved with chloroform and stored at 4°C or frozen before analysis). Analyses were performed in the station's lab on the same day or the day after. Suspended solids (SS) concentrations were determined after filtration on the GF/C porous membrane and dissolved elements measured by spectrophotometry. Total ammonia nitrogen (TAN), nitrite-nitrogen (NO₂-N), and orthophosphates (PO₄-P) were analysed using an Alliance Instruments Evolution II. NO₃-N was measured with a Technicon® Autoanalyzer II. Tank water flows were measured with electronic flow-meters (Flo-Mate, Marsh-MCBirney, model 2000).

2.3.2. Fish performance parameters

Fish mortality was counted daily. At the end of each period (P1, P2, P3), biometrics were carried out in order to adjust the biomass in the tank according to the 4 studied densities. Before biometrics, fish were anaesthetized in their tank (2-phenoxyethanol, 220 ppm m⁻³). All the tank biomass was weighed and any fish in excess were removed. Fish were taken randomly and weighed (W, g) and measured at the caudal fin fork (L, mm). Performance was calculated using the following indicators:

$$(1) \text{ Daily feed intake (DFI \%)} = (F / n) / ((B_f + B_i) / 2) * 100$$

$$(2) \text{ Feed conversion ratio (FCR)} = F / (B_f - B_i)$$

$$(3) \text{ Specific growth rate (SGR \% d}^{-1}\text{)} = (\ln W_f - \ln W_i) * 100 / n$$

where: F = Feed consumption per period (g), n = number of days in a period, B_f = final biomass (g), B_i = initial biomass (g), W_{i,f} = initial and final body average weight (g) over a period.

2.3.3. Blood parameters

Cortisolemia was measured on fish held at three densities: two extreme densities (10 and 100 kg m⁻³) and an intermediate density (40 kg m⁻³). Blood was taken from the caudal vein of 10 fish (starved 24 h before sampling), anaesthetized for 3 min at 4°C seawater. Blood samples were taken within 8 min of fish capture to minimize handling stress. Serum was separated by centrifugation (15000 rpm, 5 min, 4°C) and stored at - 20°C until analysis. Cortisol was measured using commercially available solid phase 125Iodine RIA measuring the total amount of hormone in unextracted serum (Coat-A-Count Cortisol®, D.P.C. Los Angeles, CA). The sensitivity of the assay was 2ng ml⁻¹ and the intra-assay and the inter-assay coefficients of variation were 4.7 and 6.4 (n=3), respectively. Data was obtained with a Kontron Analytical MDA 312 and analyzed using RIA software.

Proteinemia was measured on fish held at three different densities (10, 40 and 100 kg m⁻³). Proteinemia was determined with an auto analyser Max Mat Hycel, based on principle of the Biuret reaction. Bovine serum albumin was used as standard and data expressed in mg ml⁻¹. Samples for cortisolemia and proteinemia were taken at the end of the experiment and at days 21 and 42 respectively.

Other blood parameters including sodium (Na⁺), potassium (K⁺), partial pressure of CO₂ (pCO₂), Total CO₂ (HCO₃⁻ + dissolved CO₂ + H₂CO₃), glucose, haematocrit (Hct) and haemoglobin (Hb) were measured on fish held at three densities (10, 40 and 100 kg m⁻³) at day 63 (end of the third period P3). Blood was sampled with heparinized syringes in the caudal vein of 5 fish per condition and in triplicate (total of 45 fish). Measurements were obtained using an Abbott i-STAT analyser (Abbot Norge AS, N-1361 Billingstad, Norway) using EC8+ cartridges corrected when necessary in relation to the temperature.

2.4. Data analysis

Statistical analyses were performed using Statistica 5.5 1999. Data normality and variance homogeneity (Hartley tests) were assessed. Performance indicators (DFI, FCR and SGR), fish length, water quality and proteinemia were subjected to a one-way ANOVA with one fixed factor (density). Cortisol data were subjected to a two-way ANOVA to determine the combined effects of density and time.

3. Results

3.1. Water quality

The daily water renewal rate was 91, 68 and 67% for the first, second and third periods respectively, corresponding to 3, 2.3 and 2.2 m³ of make up water per kg of feed.

Average water salinity, dependant on the daily supply of seawater, was 37.2 ± 1.8‰ during the 63 day experiment. The temperature was stable around 23.4 ± 0.98°C. Oxygen concentration was above saturation for all treatments, 114.4 ± 24.9%.

The pH was significantly different for the 10 kg m⁻³ treatment compared to the other fish loads ($P < 0.001$), with an average pH recorded at 3% higher for the 10 kg m⁻³ condition. The CO₂ concentration was significantly different between the 4 treatments ($P < 0.001$) being 78% higher at 40 kg m⁻³ than at 10 kg m⁻³. However, CO₂ concentrations never exceeded 20 mg l⁻¹.

SS, TAN, NO₂-N and NO₃-N concentrations remained under 60 mg l⁻¹, 2 mg l⁻¹, 2 mg l⁻¹ and 100 mg l⁻¹ respectively as recommended by Blancheton (2000). Water quality characteristics are detailed in Table 2. A significant difference between the 4 treatments was observed for TAN ($P < 0.001$) and NO₂-N ($P < 0.001$) concentrations. The TAN concentrations in 40, 70 and 100 kg m⁻³ tanks were significantly higher (47%) than those maintained at 10 kg m⁻³ and NO₂-N concentration was 5% lower. There was no significant difference in the NO₃-N ($P = 0.253$) and PO₄-P ($P = 0.269$) concentrations between all the treatments.

3.2. Fish performance

3.2.1. Biological performance

The survival rate was 99% independently of the density and the period. Half of the mortality observed was due to fish jumping out of tanks. No aggressive behaviour was observed at the different densities tested in the RAS.

The effect of density on the DFI was significant ($P = 0.031$). Up to 70 kg m⁻³ treatment, the DFI was 8% higher than the DFI at 100 kg m⁻³ (Fig. 1). At the end of the 63 day

experiment the DFI of all treatments was improved by 7%. No significant difference in the FCR ($P = 0.154$) was observed between the 4 treatments (Fig.1). The FCR improved with time for all the groups ranging from 2.37 at the beginning of the experiment to 1.68 at day 63.

The SGR was $0.81\% \text{ d}^{-1}$ for the 10, 40 and 70 kg m^{-3} treatments. It was significantly lower ($P < 0.05$) at 100 kg m^{-3} , with $0.71\% \text{ d}^{-1}$ corresponding to a 14% of decrease (Fig. 1). The SGR increased significantly between P1 and P2 and decreased in P3 for all the density treatments (Fig. 2). Fish weight increased from $135 \pm 4\text{g}$ to $226 \pm 35\text{g}$ for the 10, 40 and 70 kg m^{-3} treatments and up to $212 \pm 34\text{g}$ for the 100 kg m^{-3} treatment.

No significant difference was detected between lengths of fish reared at the 4 different treatments at any period (data not shown).

3.2.2. Resistance to infectious challenge

The nodavirus challenge revealed no significant difference ($P < 0.05$) between treatments (ie initial densities). Mean morbidity ranged from 65 to 72% (Fig. 3) and the nodavirus was detected by ELISA in the brain of 5 affected fish from each tank, according to Varsamos *et al.* (2006). Neither morbidity nor mortality was observed in the control group inoculated with sterile supernatant culture cells (data not shown).

3.3. Blood parameter results

3.3.1. Cortisol level

No significant difference ($P = 0.71$) (Fig. 4) was identified in the plasma cortisol of the fish reared at the different density treatments (10, 40 and 100 kg m^{-3}). No change occurs over time ($P = 0.93$) or as an effect of interaction of time and density ($P = 0.96$). No significant difference was found within either density treatment or between treatments throughout the confinement period.

3.3.2. Proteinemia

At the end of P2, there was no significant difference between proteinemia of the 3 density treatments (Fig. 5), with a tendency of a higher protein concentrations for fish reared at 10 kg m^{-3} . At the end of the experiment, the proteinemia concentration of the fish reared at 10 kg m^{-3} was significantly lower than at the other densities. However, the values always remained into the range of $38\text{-}44 \text{ mg ml}^{-1}$.

3.3.3. Other blood parameters

Blood parameter measured in fish kept at 10, 40 and 100 kg m^{-3} at the end of the 63 day experiment are presented in Table 3.

Excepting the TCO_2 and associated components (PCO_2 and HCO_3), there was no significant difference between the other blood parameters of the 3 treatment groups. The TCO_2 and associated components were significantly lower in the blood of fish reared under the 10 kg m^{-3} treatment.

4. Discussion

During the whole experiment, the values of the water quality key parameters were kept close to the recommended values for sea bass aquaculture in all the density treatments (Pierce *et al.*, 1993; Person Le Ruyet *et al.*, 1997; Colt, 2006). Neither CO_2 nor TAN were above critical levels and O_2 concentration was always above saturation (Lemarié *et al.*,

2004). The average temperature was close to the optimum i.e. 24°C (Person-Le Ruyet *et al.*, 2004) and the pH was optimal.

There was no impact of density on fish mortality (1% mortality for each treatment). Density had no effect on the feed conversion ratio either contrary to the results in previous studies (Vijayan and Leatherland, 1988; Papoutsoglou *et al.*, 1998). The average FCR was high (2.37) at the beginning of the experiment, probably due to multiple fish handling and improved (1.68) through to the end of the study. There was no significant difference in the lengths of fish groups reared at different densities.

Up to 70 kg m⁻³, there was no effect of density on the daily feed intake (DFI) and on the specific growth rate (SGR). At the highest density tested (100 kg m⁻³), the DFI was significantly lower (8%) than for the other treatments as was the SGR (14%). FCR was therefore higher at the highest density although not significant. Previous studies (Baker and Aylers, 1990; Pickering, 1992; Alanära and Brännäs, 1996) revealed a correlation between the density increase and the feed intake decrease due to a stress suffered by the fish. Reduction of DFI can also be observed at low densities due to hierarchical feeding behaviour establishment (Wallace *et al.*, 1988; Jorgensen *et al.*, 1993; Zoccarato *et al.*, 1994). In our study, no aggressive behaviour was observed at different densities, even during feeding time, suggesting no hierarchical feeding behaviour being established as the density was increased. The lower SGR observed on fish reared at 100 kg m⁻³ is due to a DFI decrease related to the density. An additional reduced growth may be due to an increased metabolic demand, as suggested by Di Marco *et al.* (2008), who report an increase in serum nonesterified fatty acid concentration and the mobilization of lipid reserves in sea bass at high density.

The DFI increased slightly during the experiment (+7%), but remained lower than the DFI given by the food manufacturer (1.6% at 23°C and 1.7% at 25°C), as often observed under laboratory conditions (Di Marco *et al.*, 2008). One may consider there was an acclimatisation phase, where the fish get used to their new environment and to management conditions.

The nodavirus challenge revealed no significant difference between the 10, 40 and 100 kg m⁻³ treatments. Resistance to infection was similar independently of the density.

With regards to cortisol response to density, no significant difference was observed between in fish kept at 10, 40 and 100 kg m⁻³. Similar results were reported in sea bass reared 45 days at densities up to 21 kg m⁻³ (Di Marco *et al.*, 2003) and 45 kg m⁻³ (Marino *et al.*, 2004); mean cortisol level did not change after 2 and 6 weeks in sea bass stocked at 15, 30 and 45 kg m⁻³ (Di Marco *et al.*, 2008) even if short-term cortisol response to an acute challenge, e.g. crowding was higher at the highest density. The mean plasma cortisol level measured in our study in the different stocking density groups (33-52 ng ml⁻¹) was similar to cortisol levels reported by Marino *et al.* (2001) and by Di Marco *et al.* (2008). In other studies, extended periods of confinement at high density levels induced elevation of plasma cortisol in fish species such as salmonids (Pickering *et al.*, 1989; Mazur and Iwama, 1993), sea bream (Barton *et al.*, 2005) and ayu (*Plecoglossus altivelis*) (Iguchi *et al.*, 2003). Such discrepancies could be related to fish species and to experimental factors other than density, such as water quality variation (Ellis *et al.*, 2002). In our experiment, water quality was kept close to the recommended values for sea bass and was similar in all the density treatments.

Growth reduction is generally considered to be a good indicator of chronic stress and in some species density acts as a chronic stressor eliciting a sustained increase in plasma cortisol (Wedemeyer, 1997; Wendelaar Bonga, 1997; Procarione *et al.*, 1999). In our experiment, despite the decreased growth rate of sea bass reared at 100 kg m⁻³ from P2 onwards, no plasma cortisol increase was measured in the 100 kg m⁻³ treatment at the end of P3, indicating that reduction in growth rate observed at the highest stocking density cannot be directly explained by a mechanism involving chronic cortisol release. This study provides evidence that rearing density up to 100 kg m⁻³ is probably not a chronic stress factor for sea bass and does not result in significant changes in cortisol response. Alternatively, the lack of difference in plasma cortisol levels could reflect an

acclimatisation process to density-induced stress (by increasing cortisol turnover, cortisol deposition in the liver and/or exhaustion of inter-renal tissue) from day 21 onwards. Even if this possibility cannot be excluded, recent findings (Rotlant *et al.*, 2003) indicated that the inter-renal tissue of sea bass has a high degree of activity (higher than other species, e.g. sea bream) together with high adrenocorticotrophic hormone (ACTH) sensitivity, which is not lost after acute confinement stress at 70 kg m⁻³. The lack of significant differences in cortisol levels suggests, however, that if an acclimation process occurred it was not density-dependent, as observed in salmonids (Jorgensen *et al.*, 1993; Procarione *et al.*, 1999).

There was no significant difference between the Na, K, glucose, pH and haematocrit blood levels of the 3 density treatments followed (10, 40 and 100 kg m⁻³), but the CO₂ levels were significantly lower at 10 kg m⁻³. Schreck *et al.* (1997) observed an increase of swimming activity up to 60% higher with density increase. During this experiment, the swimming activity did not increase at 100 kg m⁻³, but the fish were more restless (Bégout, *pers. comm.*). This behaviour observed for the 100 kg m⁻³ treatment could explain the CO₂ blood levels increase.

From the middle to the end of the experiment, these proteinemia values (38-44 mg ml⁻¹) remained in the range of standard concentrations for fish (Roche and Bogé, 2000; Bayir *et al.*, 2007). These results are in accordance with the haematocrit levels which were similar for the 3 treatments at the end of the experiment. Consequently, the results cannot conclusively be considered as bearing any relation between physiological stress indicators and density in sea bass.

Conclusion

This study demonstrated, under experimental conditions and controlled water quality, that sea bass can be reared in a recirculating aquaculture system up to 70 kg m⁻³ without performance and welfare degradation. Daily feed intake and specific growth rate decrease at 100 kg m⁻³ probably due to an increase in the fish agitation. This hypothesis will have to be confirmed in further experiments. At 100 kg m⁻³, specific growth rate was 14% lower than at the second highest density (70 kg m⁻³).

Furthermore, blood parameters (except CO₂ concentration), stress indicators (cortisol, proteinemia) and resistance to infection did not differ between density treatments, confirming that high density is not a chronic stress factor for sea bass.

This study confirms that fish can be reared under high densities in RAS without performance and welfare degradation, provided that the water quality is maintained above the safe levels for aquaculture. From the farmer's point of view, the stocking density limit can be established at approximately 70 kg m⁻³ to ensure a maximal specific growth rate of sea bass.

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Tables

Table 1. Incoming water flows according to fish densities

Density kg m ⁻³	Saturated water flow m ³ h ⁻¹	Super-oxygenated water flow m ³ h ⁻¹	Total water flow	
			m ³ h ⁻¹	L kg ⁻¹ of biomass h ⁻¹
10	0.35	0.15	0.5	50
40	0.35	0.65	1	25
70	0.55	1.2	1.75	25
100	0.7	1.8	2.5	25

Table 2. Rearing water characteristics measured during the 63 day experiment according to the density (10, 40, 70 and 100 kg m⁻³); results are presented as mean ± SD. Means in the same line not sharing a common letter were significantly different ($P < 0.001$).

Water quality parameters	Density (kg m ⁻³)			
	10	40	70	100
pH	7.23 ± 0.22a	7.05 ± 0.23c	7.03 ± 0.23c	7.02 ± 0.23c
CO ₂ (mg l ⁻¹)	7.3 ± 2.6a	12.7 ± 3.7c	13.6 ± 4.8c	13.0 ± 5.1c
TAN (mg l ⁻¹)	1.03 ± 0.48a	1.44 ± 0.43c	1.53 ± 0.39c	1.57 ± 0.43c
NO ₂ -N (mg l ⁻¹)	1.55 ± 0.75a	1.48 ± 0.75c	1.46 ± 0.74c	1.46 ± 0.73c
NO ₃ -N (mg l ⁻¹)	12.60 ± 3.43a	12.60 ± 3.48a	12.42 ± 3.25a	12.37 ± 3.30a
PO ₄ -P (mg l ⁻¹)	1.24 ± 0.38a	1.25 ± 0.37a	1.25 ± 0.37a	1.23 ± 0.37a

Table 3. Blood parameters after a 63 day exposure to different fish densities (10, 40 and 100 kg m⁻³) in a recirculating system. Means in the same line not sharing a common letter were significantly different ($p < 0.05$).

	Stocking density treatment (kg m ⁻³)		
	10 kg m ⁻³	40 kg m ⁻³	100 kg m ⁻³
Na (mmol l ⁻¹)	161 ± 7.1a	158 ± 6.6a	159 ± 43.8a
K (mmol l ⁻¹)	6.4 ± 0.8a	6.6 ± 0.4a	6.5 ± 0.4a
T CO ₂ (mmol l ⁻¹)	15.2 ± 2.5a	20.8 ± 1.3b	20.6 ± 1.7b
PCO ₂ (mm Hg)	30.3 ± 3.1a	43.2 ± 4.0b	43.8 ± 5.1b
HCO ₃ (mmol l ⁻¹)	14.3 ± 2.5a	19.4 ± 1.2b	19.6 ± 1.7b
pH	7.3 ± 0.106a	7.4 ± 0.105a	7.4 ± 0.104a
Glucose (mg l ⁻¹)	81 ± 25a	77 ± 15a	84 ± 22a
Hct (% pcv)	22 ± 3a	21 ± 3a	21 ± 3a

Figures

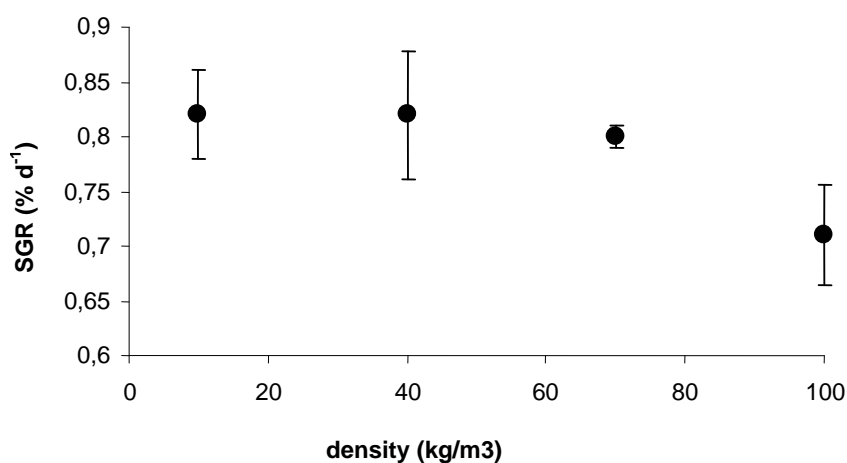
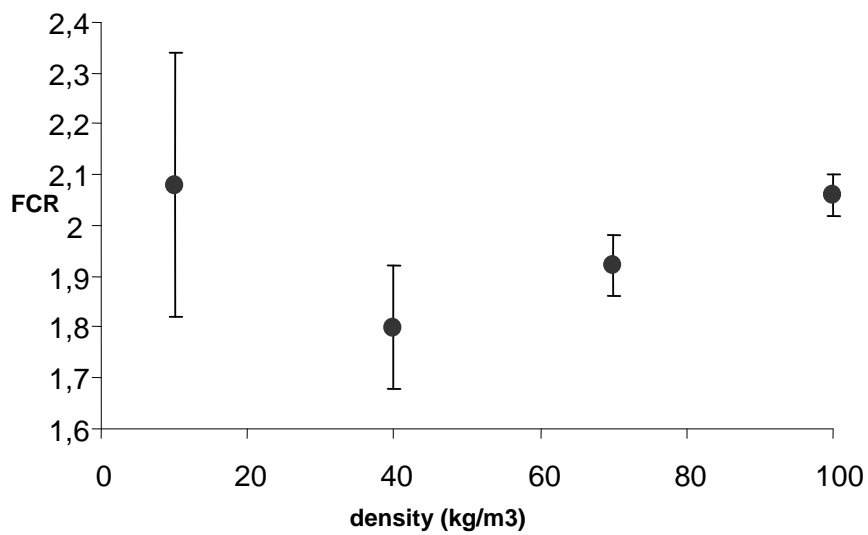
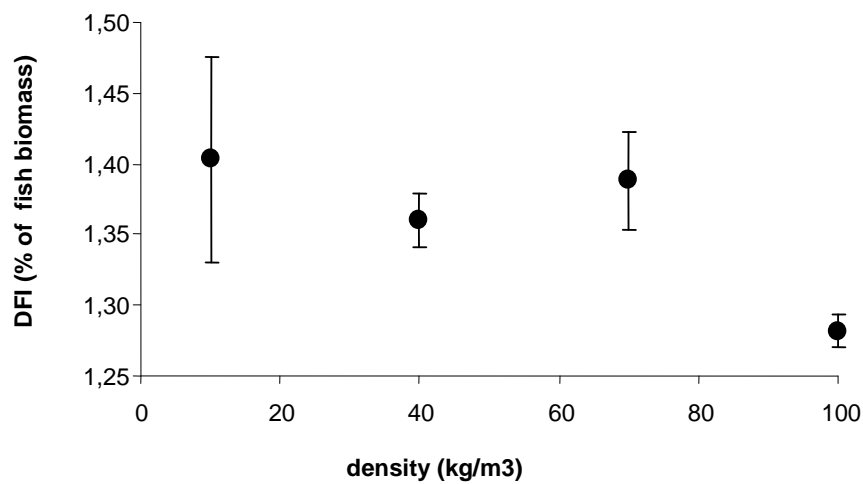


Fig.1. Daily feed intake (DFI, %), feed conversion ratio (FCR) and specific growth rate (SGR, % d⁻¹) (means ± S.D.) of sea bass reared at 4 different densities (10, 40, 70, 100 kg m⁻³) during the 63-day experiment.

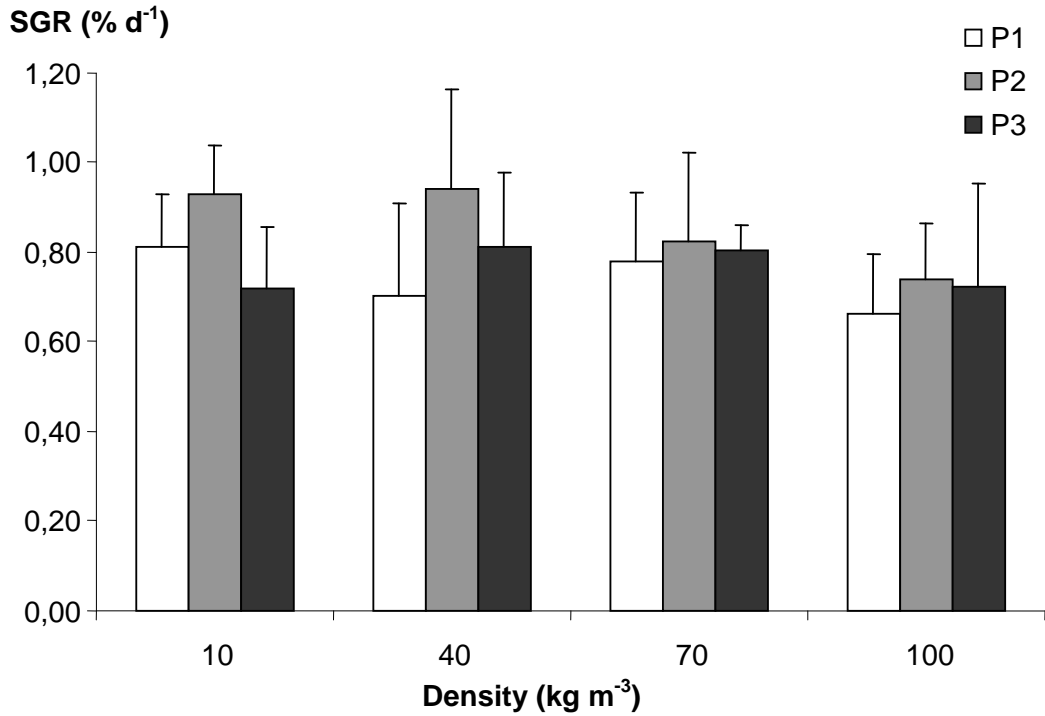


Fig.2. Specific growth rate (SGR, % d⁻¹) of sea bass stocked at densities of 10, 40, 70, 100 kg m⁻³ in tank based recirculating aquaculture system for three 21-day periods (P1, P2, P3).

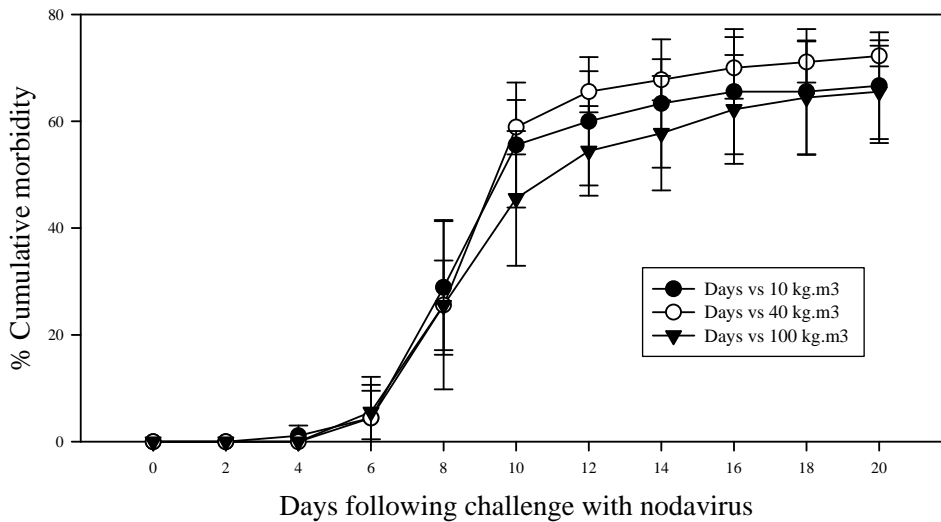


Fig.3. Cumulative morbidity (mortality and fish showing nervous symptoms) following nodavirus challenge on fish from 3 different densities (10, 40 and 100 kg.m³).

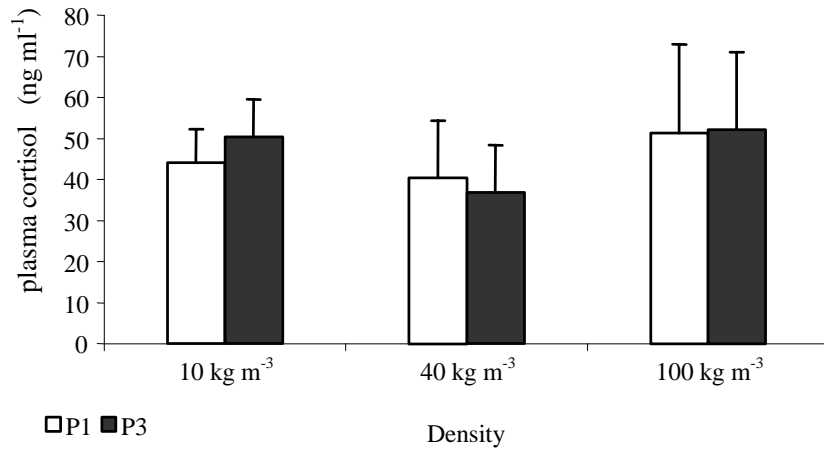


Fig. 4. Plasma cortisol concentrations (mean \pm S.E.M) of sea bass at three densities (kg m^{-3}) at the end of the first (P1, 21 day) and the third period (P3, 63 days).

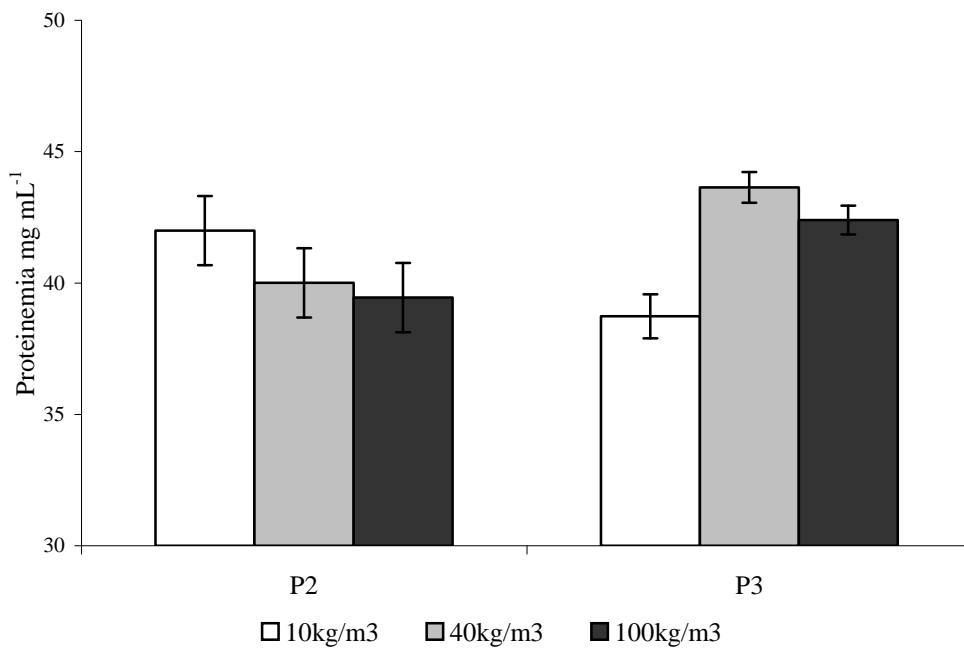


Fig. 5. Serum protein concentrations (mean \pm S.D.) of sea bass at three densities (kg m^{-3}) at the end of the second (P2) and third periods (P3).