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Effect of stocking density and feeding level on energy expenditure and stress responsiveness in European sea bass *Dicentrarchus labrax*

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ABSTRACT

European sea bass (initial weight 72 ± 4 g) were stocked in 200-L tanks at two densities: a low density (LD) ~5.5 kg m^{-3} and a high density (HD) ~36 kg m^{-3}. The tanks were part of a recirculation system and were equipped to carry out frequent oxygen measurements. At each density the fish were fed at increasing levels from around maintenance requirement up to apparent satiation. The experiment was carried out for 10 weeks at 22 °C, after which the density in the LD treatment had increased to ~10 kg m^{-3} and to ~60 kg m^{-3} in the HD treatment. At the end of the trial blood samples were taken from several fish to determine the basal levels of cortisol and glucose. Furthermore, to assess the responsiveness to an acute stressor, additional fish were subjected to individual confinement in submerged nets, blood was sampled and cortisol and glucose analysed. At the end of the trial there was no significant difference in growth performance and voluntary feed intake between the groups raised at different densities. The partitioning of energy demand for maintenance and growth highlighted a slightly higher energy maintenance requirement in the LD fish (50.9 kJ $(kg)^{0.80}$ day^{-1}) compared to the HD groups (43.15 kJ $(kg)^{0.80}$ day^{-1}). In accordance with this, an increased oxygen demand for sea bass kept at the low density was detected through weekly measurements. Analyses of the blood parameters showed, that higher stocking density resulted in higher cortisol levels in both control and stressed groups (after netting), but the effect of stocking density on the acute stress response was less pronounced at the higher feeding level.

1. Introduction

Stress in farmed fish is of considerable significance to both welfare and productivity as it has been linked to reduction in growth, abnormal behaviour and immuno-depression (Wedemeyer, 1996; Ashley, 2007). Particular attention has been drawn to stocking density as one of the key factors to influence the perceived level of stress in fish (Ellis et al., 2002; Turnbull et al., 2005; North et al., 2006).

European sea bass *Dicentrarchus labrax*, an important fish widely cultured in the Mediterranean region is mainly reared through intensive techniques in both flow-through land-based systems and sea cages with stocking densities around 35–50 kg m^{-3} (Lemarié et al., 1998; Paspatis et al., 2003). However, highly intensive recirculation systems of densities up to 100 kg m^{-3} are being developed (Blancheton, 2000).

It has been demonstrated that rearing fish at inappropriate stocking densities may impair the growth and reduce immune competence due to factors such as social interaction and the deterioration of water quality, which can affect both the feed intake and conversion efficiency of the fish (Ellis et al., 2002). However, it is not clear whether the performance of fish is influenced by sub-optimal water quality parameters associated with high densities (e.g., low oxygen level, elevated ammonia or carbon dioxide levels) or by the crowding experienced at these densities, which could cause aggressive behaviour. However these effects appear to be species-specific.

Reduced growth and feed conversion ratio during chronic stress conditions have been attributed to a change in metabolism. This effect is based on the assumption that coping with stress increases the fish's overall energy demand, which is then unavailable for growth (Wendelaar Bonga, 1997). On the other hand decreased feed consumption (Vijayan and Leatherland, 1988) social interaction (Papoutsoglou et al., 1998) and altered water quality (Pickering and Pottinger, 1987) may result in increased metabolic demands and additional expenditure of energy occurring at the expense of growth. Certainly, in the short term, the immediate metabolic cost associated with stress is a rise in oxygen demand, which is a direct measure of metabolic rate. On the other hand, stressful conditions may influence the feed intake and therefore directly the growth performance.

As a response to a possible chronic stressor such as high stocking density in European sea bass intensive culture, the following questions arise:

- What is the effect of density on feed intake, growth performance and feed efficiency?
- Is there an increase in the metabolic energy costs at high density?

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- Is there a reduction in the efficiency with which feed energy is converted into growth?
- Is density affecting the stress responsiveness of the fish?

The expected outcome of this study is to optimize the husbandry conditions of European sea bass regarding the stocking density in order to balance the demands for efficiency of production.

2. Materials and methods

All procedures involving animals were conducted in accordance with the Dutch law on experimental animals and were approved by the Wageningen University Animal Experimental Committee (DEC).

2.1. Holding facilities

The experiment was conducted in the metabolic chambers of "De Haar Vissen", Wageningen University, The Netherlands. The system used consists of 12 metabolic chambers, each sealed with a floating lid and water volume kept at 200 L by standpipes. All metabolic chambers are connected to a common recirculation system for water treatment composed of a lamella separator and drum filter to remove suspended solids, a water storage and heating tank to keep the water temperature constant, a biofilter, pumps, water refreshment unit to ensure sufficient water renewal, and trickling filter to oxygenate the water and strip off nitrogenous wastes and carbon dioxide. Water quality was monitored periodically and parameters were kept at a temperature of 21.7 °C, pH of 6.5–8.3, ammonia <2 mg.l^{-1}, nitrate <100 mg.l^{-1}, nitrite <2 mg.l^{-1}, O_2 (% saturation) >90% and salinity 23–24 ppt.

Each individual chamber was supplied with a digital water flow meter (MAGFLOW® Flowmeter MAG 5000 by Danfoss A/S, Nordborg, Denmark) and water flow in each tank was adjusted to maintain the same water quality characteristics. Two lines of sampling are connected to the inlet and outlet of each metabolic chamber, to allow online measurements of water quality parameters. The sample line from each metabolic chamber is regulated by an electromagnetic valve, which regulates the opening. The water is then conducted to a station where 3 electrodes measure values of oxygen (WTW-Trioximatic® 700 IQ, WTW GmbH, Weilheim, Germany), pH (WTW-SensoLyt DW® (SEA) 700 IQ, WTW GmbH, Weilheim, Germany) and salinity (WTW TetraCon325® 700 IQ, WTW GmbH, Weilheim, Germany). The water from each metabolic chamber was measured for 5 min, from which the measurement is based on the average value of the last 2 min and the value stored on a computer.

2.2. Fish and feeding

European sea bass obtained from France (AQUANORD, Gravelines) were used in this study. A total of 720 sea bass with an average body weight of 72 g were distributed in 12 tanks. The growth experiment lasted for 70 days. A photoperiod of 12 h daylight and 12 h darkness (12 L:12D) was maintained with day break set at 7:00 h.

Each tank was assigned a stocking density and a feeding level. Six tanks had a stocking density of 15 fish/tank or 5.5 kg m^{-3} (low density) and the remaining six tanks had a stocking density of 100 fish/tank or 36.0 kg m^{-3} (high density). At each stocking density, fish were manually fed increasing amounts of feed from low to *ad libitum* further referred to as maintenance, medium and satiation.

Fish were fed an extruded, 4 mm sea bass feed (Table 1). Diatomaceous shells (Diamol) were added as a source of acid insoluble ash (AIA) for determination of digestibility. Feed was offered twice a day at the high feeding level decreasing to once daily at the medium feeding level to ensure equal distribution of the feed pellets among the fish. To account for any uneaten feed, pellets that were flushed out were counted and multiplied by the average pellet weight. Amounts that were actually consumed are summarized in Table 2.

Table 1
Composition and proximate analysis per kg of feed (on as fed basis).

	Experimental diet
Ingredients (g kg^{-1})	
Fish meal	345
Soybean meal	200
Wheat	150
Corn gluten	85
Wheat gluten	50
Fish oil	145
Vitamin premix	14.2
DL – methionine	0.8
Diamol	10
Proximate analysis (kg^{-1})	
Dry matter, g	947
Crude protein, g	473
Crude lipid, g	191
Ash, g	89
Gross energy, MJ	21.80
Digestible protein, g	435
Digestible energy, MJ	19.45

2.3. Digestibility

Faecal samples were collected daily for digestibility analysis starting after a week the fish had adapted to the feed. The outlet of each metabolic chamber was connected to a swirl separator to efficiently collect the faeces. At the bottom of the swirl separator, a 250 mL bottle was attached where faeces settled. The bottle was kept at a low temperature (with ice) to minimize bacterial growth and protein degradation. Faecal collection was done one hour prior to feeding in the morning and in the afternoon to ensure that no uneaten feed was mixed with the faeces. Faecal matter was collected daily, centrifuged to separate salt water from the faeces and consequently freeze dried. Samples from each tank were combined over consecutive days. The apparent digestibility coefficient (ADC) expressed in percentage (%) was calculated using the following formula:

$$ADC_{\text{nutrient}} = 1 - (AIA_{\text{feed}}/AIA_{\text{faeces}} \times nutrient_{\text{faeces}}/nutrient_{\text{feed}}) \times 100$$

2.4. Energy expenditure — metabolic rate

Measurements of metabolic rate were done in two ways: 1) using direct assessments relating energy retention to energy intake, and 2) indirectly by oxygen demand.

2.4.1. Direct measurements — energy deposition

Maintenance requirements and assessment of partial efficiency for growth were carried out as per Lupatsch et al. (2001). In this approach feed is fed at increasing ration levels to obtain a linear relationship between digestible energy intake and growth in units of energy measured. The energy requirements for maintenance can be estimated by extrapolating to zero gain. The slope of the linear regression between DE intake and growth describes the efficiency of utilization of DE for growth above maintenance.

2.4.2. Indirect measurements — oxygen demand

In parallel oxygen measurements were made weekly in each tank over a 24 h period for the duration of the trial ($n = 8$ in total). Oxygen consumption was measured in each tank every 60 min during 5 min as (outlet concentration–inlet concentration) x flow rate (l min^{-1}). Daily absolute oxygen demand per tank was then calculated by adding up the demand during each consecutive hour following a 24-h cycle. Oxygen demand was then presented as the average of eight 24-h measurements

Table 2
Growth performance of *Dicentrarchus labrax* (average of two replicates).

Feeding level	Initial BW (g fish^{-1})	Final BW (g fish^{-1})	Weight gain (g fish^{-1} day^{-1})	FCR	Feed intake % day^{-1}	PRE %	ERE %
Low density							
Maintenance	73.57	74.09	0.01	–	0.33	8.95	−17.79
Medium	72.11	93.36	0.30	1.93	0.70	22.60	18.16
Satiation	71.86	129.93	0.83[a]	1.44[a]	1.21[a]	26.47[a]	35.93[a]
	73.5–70.2[b]	143–117	1.0–0.7	1.3–1.5	1.3–1	28.8–24.1	39.1–32.8
High density							
Maintenance	71.53	73.97	0.03	–	0.32	12.36	−11.01
Medium	71.08	93.35	0.32	1.79	0.68	24.39	26.51
Satiation	72.84	120.83	0.69[a]	1.43[a]	1.05[a]	28.27[a]	38.38[a]
	74.1–71.6[b]	121.7–120	0.7–0.7	1.4–1.4	1.0–1.1	27.6–29.0	41.4–35.4

[a] Values in the same column with the same letter are not significantly different ($P<0.05$).
[b] Minimum and maximum.

relative to unit of metabolic body weight (mg O_2 kg$^{-0.80}$ h^{-1}) to correct for the influence of fish size on metabolic rate (Lupatsch et al. 2003). Fish biomass at time of measurement was estimated from daily feed intake using the model for sea bass (Lupatsch et al., 2001) and verified at the final weighing. Predicted and actual final weights were in the range of ±99% agreement.

2.5. Blood sampling protocol

2.5.1. Basal values

At the end of the growth trial, ten fish from the high density treatment tanks and seven fish from the low density treatment tanks were anaesthetized (0.1 g L^{-1} of tricaine methane-sulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 0.2 g L^{-1} of sodium bicarbonate as buffer) and 1 mL of blood was collected using a hypodermic syringe from the caudal blood vessels. The blood was analysed for changes in the blood stress parameters (cortisol and glucose). Those values are used as the plasma basal levels. The collected blood was placed in cooled Eppendorf tubes containing 3 mg Na_2EDTA and mixed. The blood was centrifuged at 6000 *g* for 5 min at 4 °C. The collected plasma was stored at −20 °C for further analyses.

2.5.2. Acute stress

Additionally, another sample of ten fish from each tank of the high density treatment and seven fish from each tank of the low density treatment were subjected to an acute stress event, which consisted in placing each fish individually in a submerged net for one hour. After this the fish were anaesthetized and 1 mL of blood was collected by hypodermic syringe from the caudal blood vessels. The remaining procedure was done as described above. At the end of the experiment, all fish were killed with an overdose of anaesthesia (0.8 g L^{-1} of tricaine methanesulfonate, TMS Crescent Research Chemicals, Phoenix, Arizona, USA using 1.6 g L^{-1} of sodium bicarbonate as buffer).

2.6. Analytical methods

2.6.1. Plasma analysis

Plasma cortisol levels were measured by enzyme immunoassay (Cortisol ELISA, RE52061, IBL, GmbH). The protocol from GOD-PAP method, Roche, was used in plasma glucose analysis. The protocol from Sigma diagnostics was used in plasma lactate analysis.

2.6.2. Proximate analyses

For proximate analysis 10 fish were sampled at the start of the growth trial and 15 respectively 20 fish were sacrificed at the end from each tank. Before analysis, samples were homogenized by grinding the frozen sample in a mincing machine (Gastromaschinen, GMBH model TW-R 70, Feuma) and then freeze dried.

Identical analyses were applied for diets, faeces and body homogenates. Samples of feed and freeze-dried faecal matter were milled to pass through a 1 mm screen before analysis. Dry matter was calculated by weight loss after 24 h drying at 105 °C. Crude protein was measured using the Kjeldahl technique and multiplying nitrogen by 6.25. Crude lipid was measured after chloroform-methanol extraction. Ash was calculated from the weight loss after incineration of the samples for 24 h at 550 °C in a muffle furnace. AIA was determined by solubilising the minerals in HCl and reweighing the remaining insoluble ash. Gross energy content was measured by combustion in an IKA-C4000 adiabatic calorimeter using benzoic acid as the standard.

2.7. Calculation and statistical procedures

Feed conversion ratio (FCR) = feed intake (g feed on as fed basis)/body weight gain (g)

Feed intake (% biomass^{-1} day^{-1}) = (feed consumed day^{-1}/geometric mean weight × 100

Geometric mean = (initial BW(g) × final BW(g))$^{0.5}$

Protein retention efficiency (PRE) = protein gain/digestible protein consumed × 100

Energy retention efficiency (ERE) = energy gain/digestible energy consumed × 100

Analyses were carried out with SPSS 13.0 for Windows (SPSS Inc. 1989–2004).

The linear equations were obtained by regression analysis and optimal parameter estimates obtained with the iterative non-linear least squares algorithm of Levenberg–Marquardt. Each point in the calculation represented the combined group of fish per single tank.

Differences between groups kept at the two densities were estimated using the *t*-test. Null hypotheses were rejected at a probability level of $P<0.05$. Blood stress parameters (cortisol and glucose) and oxygen measurements were compared separately at each feeding level. The unit of observation regarding stress parameters was a single fish, whereas for the oxygen measurements the whole tank.

3. Results

3.1. Growth performance

Growth was affected by feeding intensity but was not influenced by stocking density (Table 2). Stocking density at the highest feeding level increased from 5.5 kg m^{-3} to 9.8 kg m^{-3} for the LD group and from 36 up to 60 kg m^{-3} for the HD group.

The *ad libitum* feed intake (satiation) was not significantly different between stocking densities, showing a daily feed ratio of 1.21% and 1.05% of biomass for LD and HD respectively. The same pattern was observed for FCR and protein and energy retention efficiency (Table 2).

Table 3
Body composition of *Dicentrarchus labrax* (per kg live weight, average of two replicates).

Feeding level	Dry matter g	Protein g	Lipid g	Ash g	Energy MJ
Initial	362	155	171	39	10.15
Low density					
Maintenance	352	164	137	47	9.26
Medium	356	161	147	43	9.61
Satiation	379	159	179	35	10.76
High density					
Maintenance	351	161	142	46	9.35
Medium	368	162	164	40	10.26
Satiation	387	163	184	38	11.00

Digestibility was not significantly different at the various feeding levels and was determined to be on average 92% for protein and 89.1% for energy to supply 435 g DP and 19.45 MJ DE kg^{-1} feed (Table 1).

Whole body content reflected the increased feed consumption shown by the slightly augmented levels of dry matter and energy content (Table 3). Survival was unaffected by stocking density and feeding level and was 99% at both the high and low stocking densities.

3.2. Energy expenditure

3.2.1. Direct measurements — energy deposition

Feeding sea bass graded levels of digestible energy (DE) resulted in a linear response at the respective stocking density (Fig. 1). The relationship between daily DE intake (x) and energy gain (y) referring to a metabolic weight of $kg^{0.80}$ for each density can be described by the following linear equations:

$$\text{HD} \quad y = -28.91(\pm 3.77) + 0.67(\pm 0.042)x \quad r^2 = 0.98 \qquad (1)$$

$$\text{LD} \quad y = -32.60(\pm 2.78) + 0.64(\pm 0.028)x \quad r^2 = 0.99 \qquad (2)$$

Maintenance requirement (DE_{maint}) can be found where energy gain equals zero ($y = 0$) and the efficiency of DE for growth is defined by the slope of the line (Fig. 1). The required energy intake for fish kept at the higher density is calculated from Eq. (1) as $DE_{maint} = 43.15$ kJ BW(kg)$^{0.80}$ day^{-1}. At the lower density the DE_{maint} amounted to 50.94 kJ BW(kg)$^{0.80}$ day^{-1} as calculated from Eq. (2). The efficiency of utilization of DE for growth was determined as $k_{DEg} = 0.67$ and 0.64 for the higher and lower densities respectively.

3.2.2. Indirect measurements — oxygen demand

Oxygen demand as expected increased with increasing feeding level. In line with the lower maintenance requirements (Fig. 1), respiration was significantly lower for sea bass kept at high density, and this tendency was observed at all feeding levels (Fig. 2).

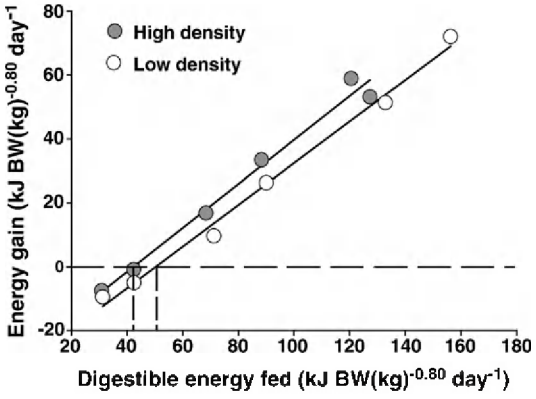

Fig. 1. Daily energy retention per unit of metabolic weight of $kg^{0.80}$ in *Dicentrarchus labrax* grown at two densities and fed increasing levels of DE.

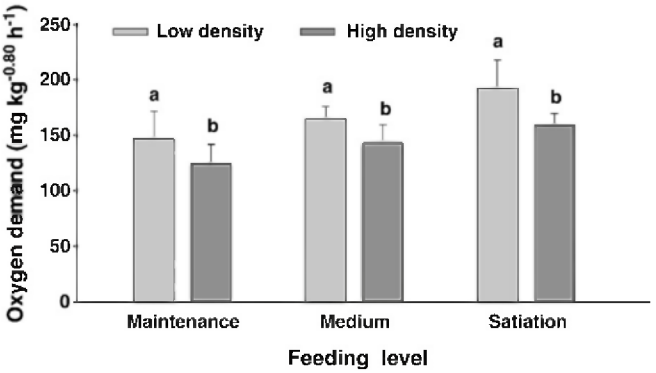

Fig. 2. Oxygen demand (mg $kg^{-0.80}$ h^{-1}) of sea bass kept at two densities and fed increasing feeding levels. (average ± STD of 8 replicate measurements, t-test was performed for each feeding level separately, values with the same letter are not significantly different ($P<0.05$).

3.3. Assessment of stress response — blood parameters

Stocking density had no significant effect on control levels of plasma glucose (before the acute stress challenge) and neither did the feeding level. There was an increase in plasma glucose levels after acute stress induction, which was independent from stocking density except at the maintenance level where plasma glucose was significantly enhanced by stocking density (Fig. 3).

Higher stocking density affected both the control levels of plasma cortisol (before the acute stress challenge) and the plasma cortisol levels after acute stress induction (Fig. 4). Cortisol levels were significantly higher in fish kept at the high density. Only in fish fed to satiation was the responding higher level to the acute stressor not significantly different.

4. Discussion

4.1. Growth performance and feed intake

Among the most important criteria for successful aquaculture are high growth rates and feed efficiency. Farmed fish are exposed to a range of potentially stressful conditions (e.g. water quality such as low oxygen, high ammonia and CO_2 levels), often in combination with high fish stocking densities, thus affecting the profitability of the aquaculture industry.

During the course of the present trial densities from 36 up to 60 kg m^{-3} did not suppress voluntary feed intake of European sea bass neither was the growth rate significantly altered (Table 2). These findings agree with the studies of other species such as the Arctic charr *Salvelinus alpinus* and the rainbow trout *Oncorhynchus mykiss*

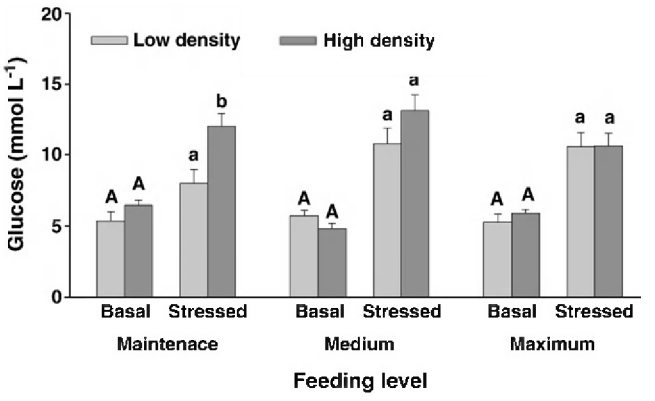

Fig. 3. Glucose content of blood serum before and after introduction of an acute stressor.

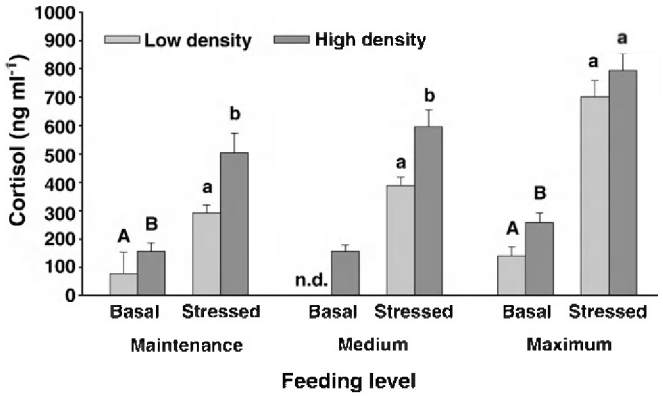

Fig. 4. Cortisol content of blood serum before and after introduction of an acute stressor.

(Jørgensen et al., 1993; North et al., 2006) where an increase in growth with increasing density of up to 120 kg m^{-3} was described. In contrast, negative effects of stocking density on growth have been reported for brook charr *Salvelinus fontinalis* (Vijayan and Leatherland, 1988; Vijayan et al., 1990) and juvenile rainbow trout (Procarione et al., 1999).

Apart from a varied response to increased stocking density among species, the effect of deteriorating water quality associated with the higher density might be one of the reasons for sub-optimal growth performance. Flow-through farming systems are subject to large fluctuations in water quality as opposed to recirculation systems where water quality parameters are controlled which facilitates the evaluation of any possible effects of stocking density separated from water quality issues as had been done in the present study.

Thus it was demonstrated by Person-Le Ruyet et al. (2008) that feed intake in rainbow trout was significantly affected by water quality, but not by stocking density per se. Also in European sea bass weight gain was found to be negatively correlated to ammonia concentrations when fed a self feeding device (Lemarié et al., 2004) and fish exposed to hypoxic conditions consumed significantly less feed thus exhibited reduced growth (Thetmeyer et al., 1999). In general, growth was correlated with feed intake, suggesting that reduced growth under less than optimal water quality conditions is primarily due to reduced appetite.

4.2. Blood parameters

In our study on European sea bass, glucose basal values were similar at both stocking densities regardless of feeding level (Fig. 3). However, basal cortisol values were significantly elevated at a density of 60 kg m^{-3} compared to a lower density of about 10 kg m^{-3} (Fig. 4), indicating a chronic stress response. Furthermore, there was a higher cortisol response in the HD group compared to the LD group due to the additional stressor (Fig. 4) even though this effect was lessened by the higher feed intake.

Slightly differing results were obtained by Di Marco et al. (2008) who reported no significant changes in glucose nor cortisol levels in sea bass, however the highest density in their study was just 45 kg m^{-3}. They also demonstrated that a stocking density of 45 kg m^{-3} did affect the fish's sensitivity to a subsequent stressor. Similarly, Ruane et al. (2002) showed that plasma cortisol responses of carp (*Cyprinus carpio*) kept at high density were elevated after a subsequent net confinement stressor compared to carp kept at low density. In line with the present study, Olsen et al. (2008) found that when subjecting Atlantic cod (*Gadus morhua*) to acute stress they observed higher plasma cortisol levels in feed deprived cod than in fed cod. Ramsay et al. (2006) identified cortisol level as an indicator of crowding stress in adult zebrafish, *Danio rerio*, however this effect only manifested itself in fasted fish, whereas in fed zebrafish, crowding did not significantly increase cortisol level.

4.3. Energy expenditure

It is commonly accepted that acute and chronic stress trigger a series of defence mechanisms that are energy demanding and therefore induce an elevation of the animal metabolic rate. However, only a few studies have attempted to estimate the extent of the additional energy requirements associated with increasing density.

Energy requirements were determined two-fold in this study and both lead to the same conclusion, that energy demands are raised in sea bass kept at the lower stocking density, despite having significantly lower cortisol levels. Gregory and Wood (1999) examined the role of cortisol itself by using implants and assessing the effects on feed intake and growth rate in rainbow trout. When compared at the same feeding levels, cortisol-treated fish had lower growth rates, reflecting a 'higher cost of living'. Contrary to this Davis and Schreck (1997) concluded from their study with juvenile coho salmon (*Oncorhynchus kisutch*), that stressed fish — stress was caused by repeated handling and chasing — experienced a 40–98% increased oxygen consumption, however exogenous cortisol implants alone did not cause any increase in oxygen demand.

By using the approach of partitioning energy demand for maintenance and growth a higher maintenance requirement of 50.9 kJ $BW(kg)^{0.80}$ day^{-1} was identified in the LD fish compared to 43.2 kJ $BW(kg)^{0.80}$ day^{-1} for the HD groups, whereas the efficiency of utilization of DE for growth above maintenance was determined as $k_{DEg} = 0.67$ and 0.64 for the higher and lower densities respectively (Fig 1). Furthermore, oxygen demands determined by repeated measurements showed significantly elevated values for fish kept at the lower density, and this outcome was repeated at all feeding levels (Fig. 2). The elevated oxygen demand was due to higher maintenance requirements as demonstrated by the ability to partition energy demand into maintenance and growth. Energy efficiency for growth above maintenance as indicated in this study is independent of stocking density. These results are in agreement with the findings of Lupatsch et al. (2003) were the partial energy efficiency was found to be independent of feeding level, water temperature and fish weight.

Social factors probably influenced the oxygen demand of the sea bass kept in the LD tanks such as increased activity due to social interference. When feeding the fish manually, individuals in the LD groups were repeatedly observed biting and chasing other specimens, and by this disturbance the metabolic rate might have been increased.

According to Jobling (1985) low density encourages the establishment of hierarchies, which may lead to a reduced feeding by low ranking fish and result in suppression of growth in those individuals. Similar findings were reported where fish under social stress had oxygen consumption rates similar to those fish forced to swim at moderate speed (Christiansen et al., 1991). High levels of locomotory activity have been shown to cause elevated metabolic rates as measured in rainbow trout (Cooke et al., 2000). In the latter study however the activity level of fish increased with increasing density.

In fish, that naturally exhibit schooling behaviour, a decrease of routine metabolism with increasing fish count has been reported. Kjartansson et al. (1988) and Papoutsoglou (1998) observed reduced agonistic behaviour at higher densities in Atlantic salmon (*Salmo salar*) and European sea bass respectively, thus lowering metabolic activity and improving feed efficiency. This is in contradiction to observations by Lefrançois et al. (2001), who did not detect any difference in the routine oxygen consumption (expressed in mg O_2 kg^{-1} h^{-1}) in rainbow trout when kept at three levels of density from lowest of 25 to the highest of 100 kg m^{-3}. Then again, the lowest density was still way above the low density of 10 kg m^{-3} from the present study.

No difference could be detected regarding the feed conversion ratio of sea bass kept at the low density (Table 2), which might have been expected considering the higher maintenance requirement. Nevertheless, proximate analyses indicated that lipid levels of whole fish body were slightly but consistently lower in fish from the LD

groups (Table 3). The increased energy demand had to be sustained by body lipid which is the major energy reserve in most fish. Therefore, we find an improved efficiency of energy utilization (Table 2) of 38.4% in fish kept at the higher densities compared to 35.9% from the low density groups.

In conclusion it can be said that European sea bass did not experience any reduction in feed intake, growth nor feed efficiency when raised at a stocking density up to 60 kg m^{-3}, neither was there any increased metabolic cost in conjunction with increased cortisol levels. Higher stocking density increased the response to an acute stressor, however this effect was dependent on feeding level and significantly reduced in well fed fish.

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