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Response to short term starvation of growth, haematological, biochemical and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) and blackspot sea bream (*Pagellus bogaraveo*, Brünnich, 1768)

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Abstract

Growth, haematological (haematocrit), biochemical (serum cortisol and glucose), and non-specific immune (lysozyme, serum haemolytic and haemagglutinating activities, extracellular respiratory burst activity) parameters, were monitored in European sea bass *Dicentrarchus labrax* and blackspot sea bream *Pagellus bogaraveo* subjected to a 31 days starvation compared to fed fish, to assess the responses to feed deprivation of these health status indicators. While haematocrit, serum cortisol, glucose and haemolytic activity of both species did not undergo significant variation following starvation, probably due to the short period applied, some non-specific immune parameters were affected significantly. In the starved sea bass, mucus lysozyme content doubled (1.8 U/mL) compared to the initial value. Haemagglutinating activity was significantly lower in starved sea bass than in fed fish after 31 days. In blackspot sea bream, a slight, not significant, reduction in haemagglutinating activity occurred 11 days after starvation. Respiratory burst activity decreased significantly in the starved fish. In spite of the limited number of examined parameters, the opportunity to use a panel of several indicators to obtain a more complete picture of health status in fish was underlined.

Keywords: Biochemical parameters; Haematological parameters; Non-specific immune parameters; Starvation; *Dicentrarchus labrax*; *Pagellus bogaraveo*

1. Introduction

Food availability may fluctuate widely in many aquatic environments; as a consequence, many aquatic animals, including fishes, may frequently experience periods of starvation. Natural starvation events can be caused by tidal, diel or seasonal cycles, long-term ecological change, and severe anthropogenic disturbance (Mc Cue, 2010). Moreover, feed restriction or deprivation for short periods may also be adopted by fish farmers as a management strategy to reduce mortality due to disease outbreaks (Shoemaker et al., 2003) or to solve water quality problems and reduce handling stress (Davis and Gaylord, 2011).

Several studies have shown that Teleostei, like other Vertebrates, are able to cope with unfavourable environmental conditions with a typical stress response (see Wendelaar Bonga, 1997). When deprived of food, animals employ various behavioral, physiological, and structural responses to cover their metabolic needs with their energy reserves; therefore these species offer interesting opportunities to study physiological adaptations to starvation (Navarro and Gutiérrez, 1995). Starvation is known to determine significant changes in the physiology of fish, particularly in their patterns of metabolic enzymes, RNA/DNA ratio and proteins (Navarro and Gutiérrez, 1995; Hung et al., 1997; Shimeno et al., 1997), the severity of which may vary depending on fish age and duration of this condition (Alliot et al., 1984; Gutiérrez et al., 1991; Echevarría et al., 1997).

The aim of this study was to understand the physiological responses to short term starvation of European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758), a species traditionally reared, and blackspot sea bream, *Pagellus bogaraveo* (Brünnich, 1768), a promising candidate species for European aquaculture diversification (Genovese et al., 2004). Blackspot sea bream rearing is, indeed, currently under consideration as an alternative to other commonly reared species due to its high commercial value, excellent taste, scarcity in the market, and adaptability to intensive farming (Micale and Patti, 1997; Genovese et al., 1998; Peleteiro et al., 2000). Although earlier studies have widely documented the effects of starvation in several fish species of interest for aquaculture industry (see Navarro and Gutiérrez, 1995; Caruso et al., 2010; Mc Cue, 2010, and references therein), a growing interest towards feed deprivation and refeeding has been recorded in fish, in relation to the compensatory growth phenomenon occurring after the application by fish farmers of short term starvation periods (Davis and Gaylord, 2011). Focusing on these features is especially required when dealing with new species, like blackspot seabream, due to the complete lack of knowledge of its metabolic traits. Physiological data on this species could provide new insights to set up suitable rearing protocols, determining the rationale for the present study. In addition, the present study focused on the behaviour of a panel of physiological parameters, including some growth (total body weight, standard length and condition factor), haematological (haematocrit),

biochemical (serum cortisol and glucose) and non-specific immune response (both humoral: mucus, plasma and kidney lysozyme, serum haemolytic and haemagglutinating activities, and cellular: extracellular respiratory burst activity) parameters, in order to monitor fish welfare both in sea bass and blackspot sea bream exposed to starvation for 31 days, considered to be representative of a brief food deprivation period.

2. Materials and methods

2.1. Animals and experimental plan

Two experiments were carried out using specimens of sea bass (*D. labrax*, Trial 1) and blackspot sea bream (*P. bogaraveo*, Trial 2) obtained both from a Sicilian fish farm. At the start of the experiments, all the individuals were subject to eye examination and were assessed for external body conditions (i.e. condition of skin, gills and fins), to exclude possible signs of impaired health status.

Both trials were performed at the experimental aquaculture plant of the Institute for the Marine Coastal Environment (IAMC) of Messina in indoor tanks of 1.9 m³, maintained under natural temperature and photoperiod. A total of eight tanks, each containing 10 fish, were used for each trial. Each tank was supplied with a constant aerated flow of seawater and provided with a complete daily water renewal; the water temperature was 20°C, salinity 38 and pH 8.2. In trial 1, sea bass had an initial mean weight ($\pm$ S.E.) of 373.1 ($\pm$ 36.4) g; in trial 2, blackspot sea bream had an initial mean weight ($\pm$ S.E.) of 738.9 ($\pm$ 47.1) g. In both trials, fish were allowed to acclimate for 2 weeks before starting the experiment; during this period they were fed until satiation a commercial diet specific for carnivorous fish, provided by Trouw Nutrition (Italy), administered once a day. The diet composition was 45% proteins, 22% lipids, 1.5% cellulose, 8% ash, 10% moisture; vitamins and mineral salts were also included.

Three days prior to initiation of the experiment, two groups each consisting of four tanks were randomly assigned to one of the two experimental treatments: 1) control, where fish were fed until

99 satiation; 2) starved, where fish did not receive any food. During the study the ethical principles
100 indicated by the European Communities Council directive (86/609/EEC) were constantly applied
101 according to a procedure conventionally adopted routinely for the treatment of living individuals in
102 our laboratory. Each experiment was carried out only once, and consisted of four samplings,
103 performed at different pre-established times (0, 11, 20 and 31 days). During each sampling, two
104 individuals were netted from each of the 8 tanks, so obtaining a total of 8 fish taken from the control
105 tanks and 8 fish from the starved ones. In total, 64 out of the initial 80 fish were collected for each
106 trial. Although this experimental design implied a reduction of the rearing density over the course of
107 the study (from 1.9 to 0.39 Kg/m³ and from 3.8 to 0.7 Kg/m³ for sea bass and blackspot seabream,
108 respectively), and could introduce a further source of variation in the physiological response of both
109 fed and starved fish, previous experiments performed on sea bass and blackspot seabream to verify
110 the effect of the progressive decrease of the stocking density on the stress response showed that this
111 did not affect significantly the levels of serum cortisol and glucose (*unpublished data*). During the
112 experiments, the fed tanks were cleaned to ensure that no food residuals or land could provide
113 nourishment and interfere with the interpretation of the results. In both trials, the sampled fish were
114 anaesthetized with 0.1g/l of MS-222 (Tricaine methanesulfonate, Sigma-Aldrich Italy), and
115 immediately the blood withdrawal was performed. Sampling procedure and time intervals were
116 selected on the basis of literature concerning the stress response in many fish species (e.g.
117 Maricchiolo et al., 2008, 2011). To avoid an induced stress response, namely to reduce possible
118 variation in haematological parameters associated to sampling, withdrawal was carried out
119 simultaneously by many operators and the time lapse between dip net introduction and withdrawal
120 lasted less than 3-4 minutes per group. Possible diurnal variations in cortisol and glucose were
121 avoided by performing all samplings at the same time of the day (i.e. 10 hours).

122 After blood sampling, fish were anaesthetized with a lethal dose of MS-222, and then killed by
123 spiking, which causes a rapid destruction of brain transmission. Each fish was weighed, the length
124 was measured and the mucus and the kidney (the last for sea bass specimens only) were collected.

2.2. Biometric measurements

The total body weight Q and the standard length L_S of each fish were measured. The condition factor K was also calculated with the formula:

$$K = Q/L_S^3 \times 100$$

where Q and L_S were expressed in g and cm, respectively.

2.3. Blood collection and treatment

The blood samples were taken separately from the caudal vein of each individual to monitor haematological, biochemical and non-specific immune defence parameters. Small volumes of whole blood were collected in heparinised tubes and used for the immediate determination of haematocrit and extracellular respiratory burst activity. The remaining blood was centrifuged and the obtained plasma was stored at -80°C for subsequent lysozyme activity measurements. Unheparinised blood samples were allowed to clot at 4°C , centrifuged at $1.500 \times g$ for 10 min, and then stored at -20°C for subsequent analysis of serum glucose, cortisol, haemolysin and haemagglutinins.

2.4. Non-specific immune response parameters

Lysozyme content was measured in mucus, plasma and kidney by the radial diffusion method (Osserman and Lawlor, 1966) in 1% agarose plates containing a 0.05% suspension of *Micrococcus lysodeikticus* (Sigma-Aldrich, Italy) as the substrate, using hen egg-white lysozyme (Sigma-Aldrich) as the standard. Lysozyme was not assayed in the kidney of blackspot sea bream because these fish were not disposed off.

Non-specific (SH50) haemolytic activity of the serum against sheep erythrocytes was determined according to Røed et al. (1992). The results were expressed in SH50 units, i.e. the reciprocal of the serum dilution giving 50% of the haemolysis.

The haemagglutinating activity of the serum against sheep erythrocytes was determined in 96-well microtiter U plates (Nunc Inc., Denmark). The haemagglutinating titre was the reciprocal of the highest dilution that showed visible agglutination.

Respiratory burst activity of whole blood was determined by measuring the extracellular production of superoxide anion using ferricytochrome C (Sigma-Aldrich) as the substrate, after treatment with Zymosan A (from *Saccharomyces cerevisiae*, Sigma-Aldrich) and phorbol-12-myristate 13-acetate (PMA, Sigma-Aldrich) as well as without any treatment (baseline values). The measurements were performed only at time 0 and at the end of the experiment.

2.5. Haematological and biochemical parameters

Haematocrit was determined in heparinized capillary tubes after centrifugation in a standard microhaematocrit centrifuge and comparison of the capillary tubes with a reference scale.

All biochemical assays were carried out using commercial diagnostic kits. Serum cortisol concentration was determined by use of an enzyme-linked (ELISA) immunoassay kit (Alpha Diagnostic International, USA). Serum glucose was determined by a kit based on the colorimetric Glucose Oxidase-Peroxidase reaction (Sclavo Diagnostics, Italy). Before its use in this study, the ELISA kit, which is originally marketed for cortisol determination in human samples, was validated in our laboratory through preliminary assays in sea bass and other marine fish species (Maricchiolo et al., 2008, 2011; Caruso et al., 2010), to verify its specificity in recognising the cortisol molecule in fish.

2.6. Statistical analysis

The results obtained for each examined parameter were expressed as mean $\pm$ S.E. per each experimental group. Data was subjected to Levene's test for homogeneity of variances and a log transformation was performed when data did not satisfy the assumption of normality. Data was first submitted to a 1-way repeated measures analysis of variance (RM-ANOVA) to assess significant differences over time. Differences between fed and food deprived groups at each sampling time were estimated using a *t-test*, considering as statistically significant only those significant at the 0.05 Probability level.

3. Results

3.1. General health condition and biometric measurements

During the starvation period, no mortality occurred either in sea bass or in blackspot sea bream tanks. Time evolution of biometric indices is reported in Table 1. In sea bass Q values increased in fed fish, while in the starved group they decreased from the beginning to the end of the experiment, although not significantly. Significantly ($P < 0.01$) lower values were recorded in the starved fish than in the fed ones, 31 days after starvation, when a weight loss of 18.39% was measured compared to fed group. In blackspot sea bream Q values slightly decreased in the starved group; conversely, in the fed fish total body weight increased, but not significantly. At each sampling time, the Q values between the fed and starved groups were not statistically different. In sea bass L_S increased significantly in the fed group (about 9.21% of the initial length) whereas in the starved one no significant changes were detected. L_S values were significantly lower in starved fish than in the fed ones 20 and 31 days after starvation. In blackspot sea bream length values were lower, although not significantly, in the starved group compared to the fed one. In both the species, the condition factor K values showed a decreasing trend during the experiment; compared to the value observed at time 0, after 31 of starvation, K decreased by a similar rate in sea bass and in blackspot sea bream (4.41 and 5.88 %, respectively).

3.2. Non-specific immune response parameters

Non-specific immune parameters measured in sea bass and blackspot sea bream yielded the values shown in Tab. 2. The lysozyme content in the mucus of sea bass was higher in the fed fish than in the starved ones. Values significantly ($P < 0.05$) higher than those recorded at time 0 were reached 31 days after starvation. In the plasma, lower lysozyme values were recorded in the starved sea bass (1.5 ± 0.1 to 2.0 ± 0.1 U/mL, respectively) compared to those measured in the fed ones (1.6 ± 0.1 to 2.1 ± 0.2 U/mL). High lysozyme values were observed in both the groups at the end of the experiment, although no significant differences were recorded between the two treatments. In the kidney, lysozyme content ranged from 2.5 ± 0.4 to 2.9 ± 0.3 U/mL and from 2.2 ± 0.3 to 2.5 ± 0.2 U/mL in the fed and starved sea bass, respectively; it increased significantly ($P < 0.05$) in fed fish

after 31 days. In blackspot sea bream, lower lysozyme values were measured both in the mucus and plasma of the starved fish than in the fed fish; statistically significant ($P < 0.01$) differences between the two groups were observed in the plasma 31 days after starvation.

The haemagglutinating titres measured in sea bass (Tab. 2) showed significantly ($P < 0.05$) lower activity in starved than in fed fish 31 days after starvation. In blackspot sea bream, the haemagglutinating titre decreased significantly ($P < 0.05$) in the starved fish after 11 days, although no significant differences were detected between the experimental groups.

In sea bass the baseline values of the respiratory burst activity were higher in fed fish compared to the starved ones (Tab.2); respiratory burst activity decreased significantly over time. The activity values increased, although not significantly, after Zymosan A and PMA treatments, suggesting a stimulating effect of these compounds on the phagocytic activity of granulocytes. At the end of the experimental period, starved sea bass showed respiratory burst activity values significantly lower ($P < 0.01$) compared to the fed fish; in this last group, PMA was particularly effective in stimulating respiratory burst activity. In blackspot sea bream the respiratory burst activity levels were initially higher in fed fish than in the starved ones, after Zymosan A and PMA treatments but not as baseline values; at the end of the experiment, respiratory burst activity values decreased significantly ($P < 0.01$), like in sea bass.

Starvation did not result in significant variations in haemolytic activity values of both fish species (not reported in Tab.2). In sea bass, from days 0 to 31, haemolytic activity values varied slightly in the starved group (from 19.1 ± 9.1 to 26.9 ± 13.0 SH50 Units), while were almost constant in the fed one (from 22.5 ± 10.1 to 25.3 ± 11.0 SH50 Units). In blackspot sea bream, haemolytic activity values were lower in the starved fish (from 0.8 ± 0.4 to 64.1 ± 12.0 SH50 Units) than in the fed ones (from 1.7 ± 0.5 to 79.6 ± 12.0 SH50 Units), increasing significantly ($P < 0.05$) over time.

3.3. Haematological parameters

The haematocrit values of both fish species were not significantly affected by starvation (data not shown). In the fed sea bass, haematocrit reached a value of 41.3 ± 4.5 % after 31 days compared to

the initial value of 31.0 ± 6.0 %; in the unfed fish, the haematocrit values measured 0 and 31 days after starvation varied between 32.3 ± 2.1 and 40.3 ± 1.8 %, respectively. In blackspot sea bream, haematocrit values decreased over time from 40.5 ± 2.8 to 34.8 ± 1.9 % in the fed fish and from 41.2 ± 3.2 to 29.5 ± 4.2 % in the starved fish, with no significant variations between the two groups.

3.4. Biochemical parameters

In sea bass, from days 0 to 31, cortisol values varied between 116.4 ± 23.0 and 139.1 ± 9.0 ng/mL in the fed fish and from 110.7 ± 27.0 to 189.8 ± 11.1 ng/mL in those unfed; starvation had no significant effects on cortisol levels. A similar result was found in blackspot sea bream, where cortisol ranged from 127.6 ± 20.5 to 208.5 ± 18.1 ng/mL and from 185.7 ± 39.3 to 256.5 ± 21.2 ng/mL in fed and starved fish, measured on days 0 and 31, respectively.

Glucose concentrations ranged from 115.9 ± 22.1 to 167.5 ± 28.0 mg/dL and from 113.7 ± 21.3 to 127.8 ± 18.1 mg/dL, in the fed and starved sea bass, measured on days 0 and 31, respectively. In blackspot sea bream, they varied over time between 59.6 ± 33.9 mg/dL and 105.3 ± 47.8 mg/dL in the fed fish, while in the starved ones they ranged from 99.1 ± 29.7 to 108.9 ± 24.1 mg/dL. For both the species, *t-test* did not show any significant difference between the fed and starved fish.

4. Discussion

The physiological response of European sea bass and blackspot sea bream to a short period of experimentally induced starvation was investigated in this study through a panel of biometric, haematological, biochemical and immunological parameters used as indicators of fish health according to the recommendations given by the European Commission (EC, 2002) on the welfare of captive animals. After feed deprivation many fish species, among which European sea bass (Şahin et al., 2000) exhibit compensatory growth when normal feeding is restored; this is the reason why increasing attention has been addressed towards the physiological alterations induced by short starvation periods, generally ranging from a few days to 4 weeks (Davis and Gaylord, 2011).

The effects of starvation in fish have been investigated by many studies, most of them focusing on the metabolic and haematological parameters. In sea bass, a short period of starvation (21 days)

leads to an intense mobilization of liver glycogen and triglycerides associated with the hepatosomatic index decrease and strong weight loss in fry (Alliot et al., 1984) as well as in juveniles (Gutiérrez et al., 1991), while in 50-days fasted adults intestinal lipid reserves depletion and plasma proteins decrease were observed, without significant muscle composition changes (Echevarría et al., 1997). Conversely, for blackspot seabream this study is the first one where the responses to starvation have been investigated through an integrated approach, which takes into account also the changes in immune defense system. Experimentally induced starvation could be compared to a condition of “chronic exposure” to stressors, which can lead to decreases in growth, disease resistance and other physiological characteristics (Wedemeyer et al., 1990).

Biometric measurements showed that weight loss occurred in both starved sea bass and blackspot sea bream, although it was significant in sea bass only after 31 days of starvation. Standard lengths values decreased significantly in sea bass 20 days after starvation. Short periods (1-2 months) of starvation had a limited effect in different fish species (Foster and Moon, 1991; Navarro and Gutierrez, 1995; Bélanger et al., 2002).

Among the non-specific immune response parameters, lysozyme doubled its activity values in the mucus of sea bass 31 days after starvation, while no significant effects were observed in plasma and kidney. The values recorded during the experiment were in the same order of magnitude as those reported in the plasma (range: 1.58 to 2.45 U/mL) of sea bass under chronic stress due to off-shore cage rearing (Caruso et al., 2005). In starved blackspot sea bream, lysozyme content was lower than in the fed fish. Activation of the immune system (Fletcher and White, 1973; Studnička et al., 1986) as well as stress (Demers and Bayne, 1997) were reported to result in increased values of lysozyme; however, enhancement or depression of lysozyme activity may occur depending on fish species, age, size and sex of the organisms, type of the examined tissue, as well as on environmental factors (Bowden, 2008).

In the present study, the haemagglutinating titres were significantly lower in the starved sea bass 31 days after starvation than in the fed fish. In blackspot sea bream, except for a slight decrease recorded 11 days after starvation, the haemagglutinating activity was unaffected by starvation.

Concerning sea bass, the spontaneous haemolytic activity did not show a clear response to starvation and its values fall in a magnitude order comparable to a previous study (Caruso et al., 2005, average SH 50 values: 6.48-55.44 SH50 Units). Conversely, in starved blackspot sea bream the SH50 values were always lower than in the fed fish, indicating the species-specificity of the immune response. Haemolytic activities decreased also in other fish species subjected to food shortage (*Onchorynchus kisutch*, Sakai, 1983; *Anguilla anguilla*, Caruso et al., 2010), as well as to chronic stress conditions (i.e. in sea bass in rearing cages, Caruso et al., 2005; in gilthead seabream *Sparus aurata* under crowding, Tort et al., 1996; Montero et al., 1999).

Besides humoral immunity parameters, also cellular immunity parameters have been reported to be suitable indicators of immune status of fish exposed to stress (Yin et al., 1995; Ortuño et al., 2001).

In both sea bass and blackspot sea bream, the respiratory burst activity showed lower baseline values in all the starved fish than in the fed ones. A similar result was observed by Vazzana et al. (2002) in sea bass under confinement stress. The treatment with zymosan, which is a beta-glucan of the yeast (*Saccharomyces cerevisiae*) wall, induced an increase of respiratory burst activity both in sea bass and blackspot sea bream; the same was reported in sea bass by Vazzana et al. (2003). On the other hand, the respiratory burst activity increased in sea bass fed oligosaccharides derived from mannan (Torrecillas et al., 2007) or a diet low in fat (Sitjà-Bobadilla and Pérez-Sánchez, 1999), suggesting the relationships between the nutritional status and the immune defense system.

Both in sea bass and blackspot sea bream the treatment with PMA caused a significant increase of respiratory burst activity compared to baseline values, confirming this compound was effective in stimulating lymphocytes proliferation, in agreement with Munoz et al. (1998).

Unlike the immunological parameters, either in sea bass or in blackspot sea bream short-term starvation did not affect the haematocrit value, which has been reported to increase, decrease or not

change after food shortage (Murachi, 1959; Sano, 1962; Smirnova, 1965; Kawatsa, 1966; Larsson and Lewander, 1973; Dave et al., 1975; Caruso et al., 2010). Also serum cortisol was unaffected both in starved sea bass and blackspot sea bream. of blood cortisol following starvation, confirming what observed by White and Fletcher (1986) and Holloway et al. (1994), in contrast with significant changes reported by other studies (Sheridan and Mommsen, 1991; Sumpter et al., 1991; Blom et al., 2000; Caruso et al., 2010). The serum cortisol levels reported in unstressed sea bass in the present study (range: 116-139 ng/mL) fall in the range (15-200 ng/ml) of values reported in literature and considered as basal for sea bass (Planas et al. 1990; Roche and Bogé 1996; Cerdá-Reverter et al. 1998; Marino et al. 2001; Di Marco et al. 2008; Lupatsch et al. 2010). Moreover, short term starvation seemed to not induce significant variations in serum glucose levels in both species. In sea bass glucose never decreased below 65-70 mg/dL which is reported as the baseline value for this species (Echevarría et al., 1997; Peres et al., 1999), in contrast with the typical decrease observed during starvation (Larsson and Lewander, 1973; Dave et al., 1975; Gutiérrez et al., 1991; Gillis and Ballantyne, 1996; Friedrich and Stepanowska, 2001). All these research, however, refer to a longer starvation period; therefore, it is reasonable to hypothesise that the lack of significant changes found in our study could depend on the short time period applied. The increase of the glyconeogenesis rate, indicated as a compensation/regulation mechanism responsible for the maintenance of high glucose levels even during starvation (Navarro and Gutiérrez, 1995; Hung et al., 1997; Shimeno et al., 1997; Figueiredo-Garutti et al., 2002; Pérez-Jiménez et al., 2007), however, was not investigated during the present research.

In spite of the limited number of examined parameters, this study proved that exposure to short-term starvation in sea bass and blackspot seabream did not significantly affect both the haematological and biochemical parameters, unlike some non-specific immune parameters such as lysozyme, haemagglutinating activity, and respiratory burst activity. This result underlines the opportunity to analyse a number of parameters broader than that commonly used in physiological studies, to obtain a more complete picture of health status in fish.

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Research highlights

- > Some physiological parameters were studied in starved sea bass and sea bream
- > Haematological and biochemical parameters did not change after 31-days starvation
- > Starvation affected significantly some immune parameters only
- > A panel of physiological parameters should be used to monitor health in farmed fish

Table 1

Mean ($\pm$ S.E.) values of the main biometric parameters measured in starved *Dicentrarchus labrax* and *Pagellus bogaraveo* compared to fed fish ($n = 8$). The asterisks indicate significant (* $P < 0.05$, ** $P < 0.01$) differences with respect to time 0.

<i>Dicentrarchus labrax</i>				<i>Pagellus bogaraveo</i>			
	Total body weight Q (g)			Time (Days)	Total body weight Q (g)		
	Fed	Starved	Fed vs Starved		Fed	Starved	Fed vs Starved
0	376.28 (65.49)	369.77 (63.69)	ns ^a	0	734.15 (90.15)	743.66 (95.25)	ns
11	391.16 (75.61)	360.57 (72.64)	ns	11	747.66 (73.59)	738.00 (60.10)	ns
20	401.95 (62.15)	345.08 (63.40)	ns	20	753.66 (88.40)	700.66 (97.71)	ns
31	412.11 (41.55)	336.29 (58.93)	**	31	775.1 (78.87)	699.17 (79.33)	ns
	Standard length L_S (cm)				Standard length L_S (cm)		
	Fed	Starved	Fed vs Starved		Fed	Starved	Fed vs Starved
0	30.50 (0.50)	30.00 (0.80)	ns	0	31.60 (2.00)	31.52 (1.75)	ns
11	32.00 (1.65)	30.30 (1.32)	ns	11	31.81 (1.18)	31.61 (0.67)	ns
20	32.67 (1.61)	29.50 (1.45)	**	20	31.83 (1.15)	31.50 (0.58)	ns
31	33.31 (1.24)**	29.60 (1.32)	**	31	32.59 (1.15)	31.50 (1.65)	ns
	Condition factor K				Condition factor K		
	Fed	Starved	Fed vs Starved		Fed	Starved	Fed vs Starved
0	1.33 (0.80)	1.36 (0.81)	ns	0	2.33 (0.45)	2.38 (0.54)	ns
11	1.19 (0.45)	1.30 (0.55)	ns	11	2.32 (0.62)	2.34 (0.89)	ns
20	1.15 (0.39)	1.34 (0.43)	ns	20	2.34 (0.77)	2.24 (0.92)	ns
31	1.12 (0.33)	1.30 (0.44)	ns	31	2.24 (0.68)	2.24 (0.48)	ns

^ans, differences between fed and starved fish at each sampling time were not statistically significant.

Table 2

Mean ($\pm$ S.E.) values of lysozyme, haemagglutinating titres and respiratory burst activity values measured in starved *Dicentrarchus labrax* and *Pagellus bogaraveo* compared to fed fish ($n = 8$).

The asterisks indicate significant (* $P < 0.05$, ** $P < 0.01$) differences between groups, different letters indicate significant differences occurring over time within each experimental group

<i>Dicentrarchus labrax</i>				<i>Pagellus bogaraveo</i>			
Mucus lysozyme (U/mL)				Mucus lysozyme (U/mL)			
Time (Days)	Fed	Starved	Fed vs Starved	Fed	Starved	Fed vs Starved	
0	0.74 (0.01) ^a	0.91 (0.28) ^a	ns	1.19 (0.16) ^a	1.03 (0.07) ^a	ns	
11	0.40 (0.01) ^a	0.74 (0.1) ^a	**	1.19 (0.07) ^a	1.03 (0.07) ^a	ns	
20	0.82 (0.09) ^a	1.20 (0.37) ^a	*	1.17 (0.1) ^a	1.04 (0.1) ^a	ns	
31	1.17 (0.1) ^a	1.81 (0.2) ^a	*	1.17 (0.1) ^a	1.03 (0.06) ^a	ns	
Plasma lysozyme (U/mL)				Plasma lysozyme (U/mL)			
Time (Days)	Fed	Starved	Fed vs Starved	Fed	Starved	Fed vs Starved	
0	1.58 (0.1) ^a	1.48 (0.12) ^a	ns	1.19 (0.07) ^a	1.11 (0.12) ^a	ns	
11	2.02 (0.1) ^a	1.90 (0.19) ^a	ns	1.18 (0.01) ^a	1.11 (0.03) ^a	ns	
20	2.05 (0.31) ^a	1.90 (0.19) ^a	ns	1.22 (0.1) ^a	1.14 (0.02) ^a	ns	
31	2.10 (0.1) ^a	2.02 (0.14) ^a	ns	1.24 (0.3) ^a	1.15 (0.01) ^a	**	
Kidney lysozyme (U/mL)				Kidney lysozyme (U/mL)			
Time (Days)	Fed	Starved	Fed vs Starved	Fed	Starved	Fed vs Starved	
0	2.64 (0.28) ^a	2.50 (0.1) ^a	ns	/	/	/	
11	2.70 (0.38) ^a	2.22 (0.33) ^a	ns	/	/	/	
20	2.47 (0.49) ^a	2.40 (0.21) ^a	ns	/	/	/	
31	2.93 (0.4) ^b	2.22 (0.2) ^a	ns	/	/	/	
Haemagglutinating titres				Haemagglutinating titres			
	Fed	Starved	Fed vs Starved	Fed	Starved	Fed vs Starved	
0	8 (4) ^a	4 (2) ^a	ns	8 (4) ^a	8 (4) ^a	ns	
11	8 (4) ^a	16 (8) ^b	ns	8 (2) ^a	4 (2) ^b	ns	
20	16 (8) ^b	16 (8) ^b	ns	4 (2) ^b	4 (2) ^b	ns	
31	32 (8) ^b	8 (4) ^a	*	4 (2) ^b	4 (2) ^b	ns	
Respiratory burst activity (nmol O ₂ per 10 ⁶ granulocytes)				Respiratory burst activity (nmol O ₂ per 10 ⁶ granulocytes)			
Baseline	Fed	Starved	Fed vs Starved	Fed	Starved	Fed vs Starved	
0	0.33 (0.02) ^a	0.06 (0.01) ^a	ns	0.09 (0.02) ^a	0.11 (0.01) ^a	ns	
31	0.11 (0.01) ^b	0.02 (0.005) ^b	**	0.03 (0.01) ^b	0.04 (0.005) ^b	ns	
Zymosan A	Fed	Starved	Fed vs Starved	Fed	Starved	Fed vs Starved	
0	1.33 (0.03) ^a	0.60 (0.03) ^a	**	0.49 (0.05) ^a	0.31 (0.02) ^a	**	
31	0.37 (0.01) ^b	0.07 (0.01) ^b	**	0.22 (0.01) ^b	0.08 (0.01) ^b	**	
PMA	Fed	Starved	Fed vs Starved	Fed	Starved	Fed vs Starved	
0	4.37 (0.5) ^a	5.65 (0.5) ^a	*	1.22 (0.08) ^a	0.79 (0.05) ^a	**	
31	16.98 (0.9) ^b	0.12 (0.01) ^b	**	0.31 (0.01) ^b	0.18 (0.02) ^b	**	

ns, differences between fed and starved fish at each sampling time were not statistically significant.