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

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22 Abstract

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

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

39 1. Introduction

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

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

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

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

78

79 **2. Materials and methods**

80 *2.1. Animals and experimental plan*

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

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

97 Three days prior to initiation of the experiment, two groups each consisting of four tanks were
98 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.

125 2.2. *Biometric measurements*

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

$$128 \quad K = Q/L_S^3 \times 100$$

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

130 2.3. *Blood collection and treatment*

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

138 2.4. *Non-specific immune response parameters*

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

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

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

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

155 *2.5. Haematological and biochemical parameters*

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

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

166 *2.6. Statistical analysis*

167 The results obtained for each examined parameter were expressed as mean \pm S.E. per each
168 experimental group. Data was subjected to Levene' s test for homogeneity of variances and a log
169 transformation was performed when data did not satisfy the assumption of normality. Data was first
170 submitted to a 1-way repeated measures analysis of variance (RM-ANOVA) to assess significant
171 differences over time. Differences between fed and food deprived groups at each sampling time
172 were estimated using a *t-test*, considering as statistically significant only those significant at the
173 0.05 Probability level.

174

175 **3. Results**

176 *3.1. General health condition and biometric measurements*

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

192 *3.2. Non-specific immune response parameters*

193 Non-specific immune parameters measured in sea bass and blackspot sea bream yielded the values
194 shown in Tab. 2. The lysozyme content in the mucus of sea bass was higher in the fed fish than in
195 the starved ones. Values significantly ($P < 0.05$) higher than those recorded at time 0 were reached
196 31 days after starvation. In the plasma, lower lysozyme values were recorded in the starved sea bass
197 (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
198 2.1 ± 0.2 U/mL). High lysozyme values were observed in both the groups at the end of the
199 experiment, although no significant differences were recorded between the two treatments. In the
200 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
201 U/mL in the fed and starved sea bass, respectively; it increased significantly ($P < 0.05$) in fed fish

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

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

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

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

225 3.3. Haematological parameters

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

228 the initial value of 31.0 ± 6.0 %; in the unfed fish, the haematocrit values measured 0 and 31 days
229 after starvation varied between 32.3 ± 2.1 and 40.3 ± 1.8 %, respectively. In blackspot sea bream,
230 haematocrit values decreased over time from 40.5 ± 2.8 to 34.8 ± 1.9 % in the fed fish and from
231 41.2 ± 3.2 to 29.5 ± 4.2 % in the starved fish, with no significant variations between the two groups.

232 3.4. Biochemical parameters

233 In sea bass, from days 0 to 31, cortisol values varied between 116.4 ± 23.0 and 139.1 ± 9.0 ng/mL
234 in the fed fish and from 110.7 ± 27.0 to 189.8 ± 11.1 ng/mL in those unfed; starvation had no
235 significant effects on cortisol levels. A similar result was found in blackspot sea bream, where
236 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
237 ng/mL in fed and starved fish, measured on days 0 and 31, respectively.

238 Glucose concentrations ranged from 115.9 ± 22.1 to 167.5 ± 28.0 mg/dL and from 113.7 ± 21.3 to
239 127.8 ± 18.1 mg/dL, in the fed and starved sea bass, measured on days 0 and 31, respectively. In
240 blackspot sea bream, they varied over time between 59.6 ± 33.9 mg/dL and 105.3 ± 47.8 mg/dL in
241 the fed fish, while in the starved ones they ranged from 99.1 ± 29.7 to 108.9 ± 24.1 mg/dL. For both
242 the species, *t-test* did not show any significant difference between the fed and starved fish.

243 4. Discussion

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

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

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

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

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

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

281 Concerning sea bass, the spontaneous haemolytic activity did not show a clear response to
282 starvation and its values fall in a magnitude order comparable to a previous study (Caruso et al.,
283 2005, average SH 50 values: 6.48-55.44 SH50 Units). Conversely, in starved blackspot sea bream
284 the SH50 values were always lower than in the fed fish, indicating the species-specificity of the
285 immune response. Haemolytic activities decreased also in other fish species subjected to food
286 shortage (*Onchorynchus kisutch*, Sakai, 1983; *Anguilla anguilla*, Caruso et al., 2010), as well as to
287 chronic stress conditions (i.e. in sea bass in rearing cages, Caruso et al., 2005; in gilthead seabream
288 *Sparus aurata* under crowding, Tort et al., 1996; Montero et al., 1999).

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

291 In both sea bass and blackspot sea bream, the respiratory burst activity showed lower baseline
292 values in all the starved fish than in the fed ones. A similar result was observed by Vazzana et al.
293 (2002) in sea bass under confinement stress. The treatment with zymosan, which is a beta-glucan of
294 the yeast (*Saccharomyces cerevisiae*) wall, induced an increase of respiratory burst activity both in
295 sea bass and blackspot sea bream; the same was reported in sea bass by Vazzana et al. (2003). On
296 the other hand, the respiratory burst activity increased in sea bass fed oligosaccharides derived
297 from mannan (Torrecillas et al., 2007) or a diet low in fat (Sitjà-Bobadilla and Pérez-Sánchez,
298 1999), suggesting the relationships between the nutritional status and the immune defense system.

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

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

304 change after food shortage (Murachi, 1959; Sano, 1962; Smirnova, 1965; Kawatsa, 1966; Larsson
305 and Lewander, 1973; Dave et al., 1975; Caruso et al., 2010). Also serum cortisol was unaffected
306 both in starved sea bass and blackspot sea bream. of blood cortisol following starvation, confirming
307 what observed by White and Fletcher (1986) and Holloway et al. (1994), in contrast with significant
308 changes reported by other studies (Sheridan and Mommsen, 1991; Sumpter et al., 1991; Blom et al.,
309 2000; Caruso et al., 2010). The serum cortisol levels reported in unstressed sea bass in the present
310 study (range: 116-139 ng/mL) fall in the range (15-200 ng/ml) of values reported in literature and
311 considered as basal for sea bass (Planas et al. 1990; Roche and Bogé 1996; Cerdá-Reverter et al.
312 1998; Marino et al. 2001; Di Marco et al. 2008; Lupatsch et al. 2010). Moreover, short term
313 starvation seemed to not induce significant variations in serum glucose levels in both species. In sea
314 bass glucose never decreased below 65-70 mg/dL which is reported as the baseline value for this
315 species (Echevarría et al., 1997; Peres et al., 1999), in contrast with the typical decrease observed
316 during starvation (Larsson and Lewander, 1973; Dave et al., 1975; Gutiérrez et al., 1991; Gillis and
317 Ballantyne, 1996; Friedrich and Stepanowska, 2001). All these research, however, refer to a longer
318 starvation period; therefore, it is reasonable to hypothesise that the lack of significant changes found
319 in our study could depend on the short time period applied. The increase of the glyconeogenesis
320 rate, indicated as a compensation/regulation mechanism responsible for the maintenance of high
321 glucose levels even during starvation (Navarro and Gutiérrez, 1995; Hung et al., 1997; Shimeno et
322 al., 1997; Figueiredo-Garutti et al., 2002; Pérez-Jiménez et al., 2007), however, was not
323 investigated during the present research.

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

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507

ACCEPTED MANUSCRIPT

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.