1   Elevated temperature promotes growth and feed efficiency of farmed ballan wrasse juveniles

2   (*Labrus bergylta*)

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4   Thomas Cavrois Rogacki[1], Andrew Davie[1], Oscar Monroig[1,2], Hervé Migaud[1*]

5

6   **Addresses**

7   [1] Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9

8   4LA, Scotland, UK

9   [2] Instituto de Acuicultura Torre de la Sal, Consejo Superior de Investigaciones Científicas

10   (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain

11

12   ***Corresponding author**

13   Prof. Hervé Migaud

14   Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA,

15   Scotland, UK

16   E-mail: herve.migaud@stir.ac.uk

17

18   **Abstract**

19   The expansion of ballan wrasse farming, used as a biological control against sea lice in Atlantic

20   salmon, is constrained by the slow growth rate in the species and extended period required to

21   reach deployment size. Rearing temperature and diets are the two main growth limiting factors

22   in fish. In this study, farmed ballan wrasse juveniles were reared at 10, 13 and 16 ºC over a

23   period of 3 months and fed two different commercial diets commonly used in marine finfish,

24   Otohime S2 and BioMar Symbio. At the end of the trial, fish growth was +125, +75 and +25

25   % compared to their initial weight in 16, 13 and 10 ºC treatments, respectively. It was suggested

26   that temperatures above 16 ºC may promote growth even further. Furthermore, feed conversion

27   ratio was significantly improved in fish reared at 16 °C. However, diets did not impact on any

28   of the growth performance indicators although a significantly higher daily feed intake was

29   observed in fish fed BioMar Symbio. Importantly, no significant effects of temperature and

30   diets on mortality and condition factor were observed. No differences were found in the fish

31   (whole-body) macronutrient composition between diets. Analysis of the protein, lipid and

32   energy digestibility revealed lower apparent digestibility coefficients than normally observed

33   in marine species, suggesting the diet formulation is not optimised for the species. Finally, fish

34   reared at 10 ºC showed increased hepatosomatic index, suggesting fat storage in the liver under

35   cold temperatures. These results showed that the production cycle could be shortened by more

36   than 4 months in fish reared at 16 ºC. This could contribute to increase hatchery productivity

37   and meet demand from the salmon production sector while reducing costs associated with the

38   nursery phase although maintaining a constant high temperature would increase operational

39   costs.

40   **Keywords**: *ballan wrasse; cleaner fish; digestibility; nutrition; recirculating aquaculture*

41   *system; temperature.*

Introduction

The sea louse is one of the most damaging parasites to the Atlantic salmon (*Salmo salar*) industry and its economic impact has been estimated to be more than 700 million euros (Brooker et al., 2018a). Cleaner fish are successfully used to delouse salmon and are considered as a more environmentally friendly treatment than chemotherapeutants. The most produced and used cleaner fish is the common lumpfish (*Cyclopterus lumpus*), with their being an emphasis on its use within the northern regions of the sector due to the species enhanced performance in cold waters (i.e. <10 ºC) (Imsland et al., 2014). The ballan wrasse (*Labrus bergylta*) has a natural range that is limited to the warmer waters of the North Atlantic gulf stream (Sayer & Treasurer 1996) and is thus more favoured within the southern region of European salmon production. While ballan wrasse have been acknowledged as being highly effective at delousing salmon, the majority of currently deployed specimens remain of wild origin. However, due to the unpredictability of the fishery, the associated biosecurity risk and the sustainability concerns (Blanco Gonzalez and de Boer, 2017), the industry would favour farming of wrasse as opposed to sourcing of wild captured fish. In order to ensure a reliable, disease-free and sustainable supply of juveniles, a significant effort must be put towards closing the cycle and farming the species. However, the length of the growing cycle in captivity (up to 24 months for a deployable size of 50 g) is a limiting factor to meet increasing demands for large quantities of farmed ballan wrasse (Bolton-Warberg, 2017; Brooker et al., 2018b).

Several abiotic factors influence growth, feed intake and nutrient uptake, among which temperature and diets are the most important (Brett and Groves, 1979; Jobling, 1996). For instance, growth, expressed as the specific growth rate (SGR), increases with temperature until reaching the maximum growth potential of the species and then rapidly declines thereafter (Brett and Groves, 1979). Ultimately, the optimal rearing temperature for finfish species could be defined as the temperature that maximises growth, feeding efficiency while it preserves the

67 health and welfare of the fish. Temperature requirements are known for many marine species,

68 which results in the establishment of validated commercial rearing protocols e.g. turbot

69 (*Scophthalmus maximus*) (Burel et al., 1996, Imsland et al., 1996) and European sea bass

70 (*Dicentrachus labrax*) (Person-Le Ruyet et al., 2004). The implementation of temperature

71 optima in the hatcheries subsequently optimises growth and feed efficiency, which ultimately

72 has significant positive impact on production efficiency. In turbot, rearing fish at 17 ℃

73 compared to 8 ℃ resulted in a 50 % increase in specific growth rate (SGR) (Burel et al., 1996).

74 In seabass, rearing fish at 22 ℃ compared to 16 ℃ increased SGR by 75 % and enhanced feed

75 efficiency (Person-Le Ruyet et al., 2004). However, to date, no published data is available on

76 optimal rearing temperature for ballan wrasse and consequently no standardised rearing

77 temperature has been applied in commercial hatcheries. Ballan wrasse is naturally distributed

78 in an area covering the North East Atlantic Ocean (Sayer et al., 1996), where temperature

79 ranges (on average) from 2.5 ℃ in winter at the most northern point of their natural distribution

80 (e.g. Norwegian coast) up to 22 ℃ in summer in the most Southern regions (e.g. Portuguese

81 coast) (US Department, 1998). Although this indicates a broad temperature tolerance range,

82 species preferences are greatly dependent upon the geographical origin of a specific population

83 as demonstrated by Björnsson et al., (2007) in Atlantic cod (*Gadus morhua*). Thus, in the

84 context of ballan wrasse, production focus must be directed towards optimising environmental

85 conditions for stocks derived from the main production areas.

86   There is equally limited published data on the nutritional requirements of ballan wrasse.

87 In their natural habitat, they feed on decapods and bivalves as well as smaller amounts of algae

88 and gastropods (Deady and Fives, 1995). However, while their natural diet is not piscivorous,

89 ballan wrasse hatcheries use commercially available diets which are rich in fish meal (Hamre

90 et al., 2013; Kousoulaki et al., 2015). Such diets may not be the most suitable for ballan wrasse.

91 Furthermore, a recent study suggested that ballan wrasse prefer easily digestible ingredients

92   (e.g. krill or fish protein hydrolysate) as opposed to fish meal diets (Lie et al., 2018). At the

93   moment, there is no data on nutrient digestibility in ballan wrasse, which severely limits the

94   formulation of suitable diets for the species and the associated benefits on feed efficiency.

95   This study aimed to investigate the effects of temperature on ballan wrasse growth, feed

96   efficiency and nutrient digestibility in relation to diet. To do so, fish were reared under three

97   constant temperatures, within the typical range experienced by cultured ballan wrasse

98   populations and fed with two different commercially available diets formulated for marine

99   finfish species commonly used in ballan wrasse hatcheries.

100

101   **Materials and Methods**

102   *Experimental system and culture conditions*

103   The trial was carried out at the Machrihanish Marine Environmental Research Laboratory

104   (Machrihanish, UK). The main water supply used in the experimental system was filtered to

105   10 µm through a combination of sand and pressure filters followed by UV treatment (1.18 MJ

106   h^{-1}). The study was performed in 18 circular flat-bottom tanks (100-l) divided evenly between

107   three independent recirculation systems (TMC system 1,000: Tropical Marine Centre, UK)

108   with a water turn over in the tanks of approximately 1 hour. Tanks were illuminated 24 hours

109   with an average intensity of 0.8 W m^{-2} at the water surface. Each tank was stocked with 60

110   juvenile fish (14.7 ± 0.7 g) produced by the neighbouring commercial ballan wrasse hatchery

111   (MOWI Scotland Ltd, Machrihanish, UK) where they had previously been reared at 13 °C.

112   Fish were acclimated to the study temperatures at a rate of 0.5 ºC day^{-1} and were then

113   maintained at the following experimental temperatures (Table 1). Water quality (e.g. NH_4,

114   NO_2, NO_3, pH, salinity and O_2) was monitored daily in each RAS and no differences were

115   observed between systems (Table 1). At the beginning of the experiment, the stocking density

116   was set at 8.5 ± 0.5 kg m^{-3} and by the end of the experiment it reached 9.3 ± 0.8 kg m^{-3}, 13.3 ±

117   0.8 kg m^{-3} and 16.5 ± 1.6 kg m^{-3} at 10, 13 and 16 ºC, respectively, which remains within

118   commercial stocking density ranges.

119

120   *Feeding and digestibility study*

121   For the first 93 days, termed the "growth" trial, the fish were fed two commercial marine diets,

122   namely Otohime S2 (Marubeni Nissin Feed Co., Tokyo, Japan) and BioMar Symbio (BioMar,

123   Brande, Denmark), which are commonly used for on-growing in commercial ballan wrasse

124   hatcheries in the UK and Norway. The biochemical analysis (Table 2) showed that the diets

125   were close to isoenergetic (20.1 ± 0.0 and 19.5 ± 0.3 MJ kg^{-1} for OS2 and Symbio,

126   respectively). OS2 appeared to have a lower moisture content (6.7 ± 0.0 %) compared to

127   Symbio (8.8 ± 0.0 %). Ash, crude protein (CP) and crude lipid (CL) were higher in OS2 (14.2

128   ± 0.0 %, 54.8 ± 0.0 % and 14.3 ± 0.4 %, respectively) compared to Symbio (9.5 ± 0.1 %, 51.0

129   ± 0.1 % and 10.9 ± 0.3 %, respectively). The carbohydrate content of Symbio was higher than

130   in OS2 (28.6 ± 0.3 % and 16.7 ± 0.3 %, respectively). Finally, the carbohydrate/crude lipid

131   ratio (CHO/L) was more than two times higher in Symbio than OS2 (2.6 ± 0.1 and 1.2 ± 0.1,

132   respectively). The diets were tested in triplicate ($n = 3$) within each independent recirculation

133   system. Feeds were automatically distributed using Eheim twin-screw feeders controlled by a

134   central command unit. Throughout the experiment, all fish were fed to satiation plus excess

135   confirmed by the presence of a quantifiable number of uneaten pellets at the bottom of the

136   tanks. Feed recovery was done daily by siphoning the bottom of the tanks to collect uneaten

137   pellets which were weighed and converted into a dry weight using previously made standard

138   curves. At the end of the 93 days, the trial was extended for another 4 weeks to collect enough

139   faeces for digestibility analyses. Yttrium oxide, an inert digestibility marker, was added to

140   BioMar Symbio during the aquafeed production process at approximately 350 mg kg^{-1}. For

141   technical reasons, yttrium could not be added to OS2 and therefore, digestibility data could

142   only be obtained from Symbio fed fish. Fish were fed Symbio continuously to satiation for 2

143   days before turning off the feeders and flushing the tanks to remove the faeces and uneaten

144   food. On the 3rd day, the freshly produced faeces were collected by siphoning every 2 hours,

145   and storing samples frozen at -20 ℃. The procedure was repeated until enough faecal material

146   was collected for nutritional analysis (*circa* 28 g of wet faeces per tank).

147

148   *Sampling*

149   The sampling regime during the growth trial included an initial (D0), interim (D45) and final

150   (D93) sample points for which the biomass (g) and the total number of fish per tank were

151   recorded. Also, the total length (cm) and weight (g) of 20 fish per tank were recorded and

152   allowed the calculation of K factors using the following equation:

153   $K = 100 \times (\text{weight, g}) / (\text{total length, cm})^3$

154   Individual weight (g), feed conversion ratio (FCR), specific growth rate (SGR), thermal growth

155   coefficient (TGC) and daily feed intake (DFI) were calculated as follow using the biomass data:

156   Individual weight (g) = biomass (g) / number of fish

157   FCR = (feed intake, g) / (biomass gain, g)

158   $\text{SGR (\% day}^{-1}) = 100 \times (\ln (\text{final biomass, g}) - \ln (\text{initial biomass, g})) / (\text{time, days})$

159   $\text{TGC} = 1000 \times ((\text{final biomass, g})^{1/3} - (\text{initial biomass, g})^{1/3}) / (\text{sum degree days, ℃})$

160   $\text{DFI (\% days}^{-1}) = \text{FCR} \times \text{SGR}$

161   At the end of the growth trial, six fish per tank were sacrificed, pooled (two pools of three

162   fish per tank) and preserved at -20 ℃ for proximate composition analysis. Another six fish

163   were sacrificed, and their livers weighed to calculate the hepatosomatic index (HSI = 100 x

164   (weight liver, g fish^{-1}) / (body weight, g fish^{-1})). The livers were then pooled (two pools of three

165   livers per tank) and preserved at -20 ℃ for lipid composition analysis.

166

Proximate composition

Proximate composition of the feeds (Table 1) and fish carcasses were determined according to standard procedures (AOAC, 2000) in place at the Nutrition Analytical Service laboratory of the Institute of Aquaculture (Stirling, UK). All samples were analysed in technical duplicates ($n = 2$). Before analysis, feed samples were ground with a mortar and pestle while the carcasses were homogenised in a blender (Waring Laboratory Science, UK) to produce a paste. Moisture content was calculated after drying weighed samples in an oven at 110 °C for 24 h and ash content determined after incineration of a weighed sample at 600 °C for 16 h. Crude protein content was measured by determining N content (N × 6.25) using automated Kjeldahl analysis (Tecator Kjeltec Auto 1030 analyser; Foss). Energy content was measured using bomb calorimetry calibrated with benzoic acid (Gallenkamp Autobomb; Gallenkamp & Co. Ltd). Crude fibre in the diets was measured after de-fattening the samples in petroleum ether and digestion in 1.25 % sodium hydroxide followed by a digestion in 1.25 % sulphuric acid (Fibercap system, Foss). Crude lipid content was measured by extraction of the total lipids by homogenisation in chloroform/methanol (2/1, v/v) according to Folch et al. (1957). Fatty acid methyl esters (FAME) analyses of diets and liver samples were prepared according to Christie (2003) from total lipids by acid-catalysed transesterification at 50 °C for 16 h. FAME were separated and quantified by GLC using a Fisons GC-8160 (Thermo Scientific) equipped with a 30 m × 0.32 mm internal diameter × 0.25 μm ZB-wax column (Phenomenex), on-column injector and a flame ionisation detector. Data were collected and processed using Chromcard for Windows (version 2.01; Thermoquest Italia S.p.A.).

Faeces collected during the digestibility study were freeze-dried for 72 hours and homogenised. Proximate composition was assessed similarly to that of the diets and carcasses. To determine their yttrium oxide content, Symbio diet and faeces were digested in 69 % nitric acid in a microwave (MARSXpress, CEM) for 40 min (20 min ramping to 120 ºC and 20 min

192 holding that temperature). Digests were transferred into a volumetric flask and made up into x

193 25 dilutions with distilled water. Samples were analysed by Inductively Coupled Plasma Mass

194 Spectrometry (ICP-MS, Thermo Scientific Model X Series 2, USA). Apparent digestibility

195 coefficient (ADC) was calculated as follow:

196 $ADC_{nutrient}$ (%) = [1 − (Nutrient$_{faeces}$, %) / (Nutrient$_{diet}$, %) x (Yttrium$_{faeces}$, mg kg^{-1}) /

197 (Yttrium$_{diet}$, mg kg^{-1})] x 100

198

199 *Statistics*

200 All data are presented as mean ± standard deviation (SD). Percentage data were subjected to

201 arcsine square-root transformation prior to statistical analyses. Normality and homogeneity of

202 variance in the data were confirmed using Shapiro-Wilk and Levene's tests, respectively.

203 Growth indicators and nutritional data were analysed by two-way ANOVA using

204 "Temperature" and "Diet" as factors, followed by Tukey's post-hoc test when relevant.

205 Mortality data was not normally distributed and was therefore analysed using the non-

206 parametric tests Kruskal-Wallis and Mann-Whitney U for treatments temperature and diets,

207 respectively. Apparent digestibility for fish fed Symbio was analysed by one-way ANOVA

208 followed by Tukey's post-hoc test. All treatment effects were considered significant at a

209 significance level of $P<0.05$. To test for isometry in growth rate the weight-length relationship

210 (WLR): $W = aL^b$, where W is the body weight, L the standard length and a and b are parameters

211 of the relationship was transformed into its logarithmic equivalent: log(BW) = log(a) +

212 b*log(FL) for analysis by least-square regression based (Froese, 2006). Significant variations

213 from the isometry (slope = 3) were determined using a Student *t*-test (α = 0.05) (Arslan et al.,

214 2004). All data were analysed using SPSS (IBM SPSS Statistics 23, NY, USA) and Microsoft

215 Excel (v16, WA, USA).

216

217

Results

Growth, feed intake and survival

While all populations began the study were of a statistically comparable size, at the end of the 93-days growth period, the increasing temperature resulted in significantly greater length, ranging from 10.4 ± 0.3 to 10.5 ± 0.2 cm when reared at 10 ºC (OS2 and Symbio, respectively), 11.8 ± 0.2 to 11.5 ± 0.2 cm when reared at 13 ºC (OS2 and Symbio, respectively) and 12.3 ± 0.3 cm when reared at 16 ºC (both diets) (Table 3, Fig. 1A). Fish weight was also significantly greater at higher temperatures, with a 134 % increase in fish reared at 16 ºC and fed OS2 compared to their initial weight and 128 % increase in fish fed Symbio at the same temperature (Table 3, Fig. 1B). Fish reared at 13 and 10 ºC increased weight by *circa* 73 % and 25 %, respectively with there being no differences in relation to the diet fed. The WLR and least-square regression line based on individuals farmed at different temperatures showed that, independently from the diet, fish reared at 16 ºC followed a positive allometric growth relationship while fish reared at 10 and 13 ºC followed an isometric growth (Fig. 2). Over the growth period, SGR significantly increased with increasing temperature from 0.2 % day^{-1} at 10 ºC to 0.5 % day^{-1} at 13 ºC and 0.8 % day^{-1} at 16 ºC with there being no effect in relation to diets within temperatures (Table 3, Fig. 1C). TGC followed the same pattern than SGR with 0.2 at 10 ºC, 0.4 at 13 ºC and 0.5 at 16 ºC and again there being no effect in relation to diet within temperatures (Table 3).

There was a differential response in DFI between diets in relation to temperature (Table 3). DFI in fish fed OS2 did not significantly differ between temperatures, varying from 0.9 ± 0.2, 1.2 ± 0.0 and 1.0 ± 0.1 % day^{-1} at 10, 13 and 16 ºC, respectively. On the other hand, DFI in fish fed Symbio significantly increased from 1.1 ± 0.0 to 1.4 ± 0.0 % day^{-1} between 10 and

241   13 ℃ and remained elevated at 16 ℃. Overall, DFI was significantly higher at 16 ℃ in fish

242   fed Symbio compared to those fed OS2 (Fig. 1D).

243   There was no impact of diet within a given temperature on observed FCR. Significant

244   differences in FCR were observed in relation to temperature with reduced FCR in fish reared

245   at 16 ℃ (1.4 ± 0.1 and 1.8 ± 0.3 for fish fed OS2 and Symbio, respectively) and 13 ℃ (2.3 ±

246   0.1 and 2.7 ± 0.5 for fish fed OS2 and Symbio, respectively) compared to 10 ℃ (4.8 ± 1.0 and

247   5.1 ± 0.9 for fish fed OS2 and Symbio, respectively) (Table 3, Fig. 1E).

248   No differences in K were found between treatments (Table 3). Mortality rates were not

249   significantly affected by treatments, ranging from 4.6 ± 2.1 to 10.1 ± 1.6 % at the end of the

250   experiment (Table 3). Hepatosomatic index (HSI) was significantly higher in fish reared at 10

251   ℃ (1.35 ± 0.16 and 1.39 ± 0.38 % for OS2 and Symbio, respectively) than at 16 ℃ (0.64 ±

252   0.12 and 0.71 ± 0.08 % for OS2 and Symbio, respectively) (Table 3, Fig. 1F).

253

254

255   *Proximate analyses*

256   Moisture and ash contents were significantly higher in fish reared at 10 ℃ compared to 16 ℃

257   (Table 4, Fig. 3A and 3B). Crude protein content in fish was not affected by any treatments but

258   appeared to be lower than reported in wild ballan wrasse (mean of 66.4 ± 1.2 and 71.1 ± 3.9

259   %, respectively) (Table 4). Crude lipid levels were significantly higher in fish reared at 16 ℃

260   (13.8 ± 1.7 and 13.1 ± 2.3 % for OS2 and Symbio, respectively) compared to 10 ℃ (7.9 ± 0.8

261   and 8.6 ± 0.3 % for OS2 and Symbio, respectively) (Fig. 3C). CL levels reported in wild fish

262   (12.8 ± 5.7 %) appeared to be comparable to that observed in 13 ℃ and 16 °C treatments.

263   Carbohydrates were significantly lower in fish reared at 13 ℃ (4.7 ± 0.7 and 4.1 ± 0.2 % for

264   OS2 and Symbio, respectively) compared to 10 ℃ (5.8 ± 0.8 and 6.4 ± 0.7 % for OS2 and

265   Symbio, respectively) (Fig. 3D). Carbohydrates appeared to be lower at 16 ℃ (4.5 ± 0.9 and

266   4.5 ± 2.1 % for OS2 and Symbio, respectively) compared to 10 °C, but not significantly (Fig.

267   3D). No carbohydrate data is available for wild ballan wrasse.

268   In terms of macronutrient uptake, digestibility analysis could be done in Symbio due to

269   the inclusion of yttrium oxide in this diet. Overall, ADC levels were low (i.e. <76 %) (Table

270   4). The increase of the rearing temperature from 10 ºC to 13 ºC resulted in an apparent reduction

271   in digestibility coefficient of proteins ($ADC_{Proteins}$), lipids (ADC_{Lipids}) and energy (ADC_{Energy})

272   although this was not significant at the $P<0.05$ level (one-way ANOVA, $P = 0.063$, $P = 0.134$

273   and $P = 0.084$ for $ADC_{Proteins}$, ADC_{Lipids} and ADC_{Energy}, respectively).

274   The lipid content of the liver remained constant between treatments (mean of 9.6 ± 2.8

275   %) although a decreasing trend at lower temperature was observed (Table 5). Liver of fish fed

276   Symbio displayed a higher content of linoleic acid (18:2n-6) (~+97 % across temperature

277   treatments) and n-6 PUFA (~+68 % across temperature treatments) (Table 5).

278

279   **Discussion**

280   The salmon industry is currently seeking to increase the number of farmed deployed ballan

281   wrasse to tackle sea lice outbreaks and reduce pressure on wild stocks. However, the production

282   of this new aquaculture species is hampered by its slow growth, which is attributed to intrinsic

283   characteristics of the fish but also to the lack of understanding of the species environmental

284   and nutritional preferences. As reported in many temperate marine fish species, an increase in

285   the rearing temperature can promote growth until it reaches a plateau before then rapidly

286   declining (Jobling, 1996). Wild ballan wrasse can live in a wide range of temperatures as

287   experienced in their natural habitats (e.g. 6 to 17 ºC in Scotland, Marine Scotland, 2011) and

288   no standardisation between commercial hatcheries have been applied yet with water

289   temperature during juvenile rearing ranging from 8 to 16 °C (Featherstone, P.; Barge, A.;

290   personal communication). Results from the present study clearly showed enhanced growth

291 performance (i.e. weight gain, SGR, TGC) of ballan wrasse juveniles reared at a constant

292 temperature of 16 °C. Ideally, the study should have normalised sampling times with degree

293 days. However, this would have extended the study by 56 days and led to stocking densities in

294 the 16 °C treatment exceeding accepted limits. Therefore, it was decided to limit the study to a

295 fixed number of days with a minimum of doubling of fish weight in the upper temperature

296 treatment.

297 The Weight-Length Relationship (WLR) analysis showed that fish reared at 16 ºC displayed a

298 positive allometric growth as reported previously in a mixed-gender population of wild ballan

299 wrasse exposed to similar natural temperatures (Leclercq et al., 2014b). However, under

300 hatchery conditions, growth pattern appeared to change to isometric in fish reared at 10 and 13

301 ºC. It is known that for teleosts such as Atlantic salmon, heavier individuals tend to exhibit a

302 positive allometric relationship often resulting from a higher condition factor K (Leclercq et

303 al., 2010). Although in the present study no differences in K factors were observed across

304 treatments, data showed that ballan wrasse somatic growth and subsequent weight gain was

305 enhanced at 16 °C compared to that of lower temperatures, resulting in heavier fish hence a

306 positive allometric growth. In production, farmers should closely monitor the condition of their

307 fish, particularly when reared at 16 °C, so any change in fish condition (i.e. increase of K above

308 normal values) can be detected and mitigated if possible, through nutritional interventions.

309 A significant difference in fish length between rearing temperatures was also observed at the

310 end of our experiment suggesting that higher temperatures promote somatic growth as well as

311 skeleton development (Schneider et al., 2000). Condition factor was not affected by either

312 treatment and remained within normal levels for the species according to Skiftesvik et al.

313 (2013) and Leclercq et al. (2014a). In those studies, K of ballan wrasse juveniles coming from

314 a hatchery environment was higher (≥1.5) than for wild fish or fish that had been deployed for

315 at least 6 weeks (<1.5). Under farming conditions, fish are fed *ad libitum* aquafeeds, whereas

316 food can be a limiting factor in the sea-cages or the wild. Furthermore, they can exhibit a

317 reduced swimming activity in rearing tanks compared to their natural environment, thus

318 resulting in a higher condition factor, mirroring the study from Skiftesvik et al. (2013).

319 Both TGC and SGR showed no difference due to diets and thus the pooled dataset demonstrated

320 a constant increase suggesting that the temperature at which maximum growth is obtained for

321 the species lies beyond 16 °C. While DFI did display a differential response in relation to diet,

322 in both cases DFI reached a plateau after 13 °C (i.e. 1.2-1.4 %), suggesting the maximum feed

323 intake for the species was reached. Fish fed Symbio displayed a higher DFI compared to those

324 fed OS2. This may be due to the higher moisture level in Symbio than in OS2 which may have

325 increased diet palability as suggested previously (Helland et al., 2013). As mentioned earlier,

326 the culture of ballan wrasse is very recent hence the literature surrounding the nutritional

327 aspects of the species is very limited. To our knowledge, this is the first published study

328 showing data on feed efficiency indicators (i.e. FCR, DFI) and digestibility for farmed ballan

329 wrasse juveniles and therefore there are no comparison points besides that of data from other

330 farmed marine finfish species. FCR was greatly reduced at 16 °C (1.4) similarly to that of other

331 well-known species farmed under commercial protocols, such as European sea bass (Kaushik

332 et al., 2004; Torrecillas et al., 2017) or turbot (Van Ham et al., 2003; Cho et al., 2005). At 16

333 °C, the increased daily feed intake would be a result of a higher metabolism (Jobling, 1981;

334 Jobling, 1988). The apparent digestibility coefficient for protein ($ADC_{Protein}$), lipid (ADC_{Lipid})

335 and energy (ADC_{Energy}) were significantly lower than what is commonly observed in

336 piscivorous marine species (Mundheim et al., 2004; Glencross et al., 2007), where ADC for

337 the three macronutrients are usually found above 80 %, when using species optimised diets. In

338 our study, the $ADC_{Protein}$ ranged from 47.3 to 65.2 %, which suggests that between 34.8% and

339 52.7% of the dietary proteins were not digested thus resulting in a considerable waste of

340 resources. The same applies to ADC_{Lipid} and ADC_{Energy}, which were also quite low (42.9-54.0

341   % and 52.5-75.6 %, respectively). These results support the fact that available diets used for

342   ballan wrasse may not be fully suitable for the species. A study from Kousoulaki et al. (2015)

343   showed that the inclusion of shrimp and krill hydrolysate, both very high-quality protein

344   sources, in ballan wrasse weaning diets had a positive effect on the growth and survival of the

345   fish compared to diets containing commercial medium quality fish meal, similarly to that of

346   the present study. Although Kousoulaki's study lacked data on digestibility, it suggests that

347   alternative and high-quality ingredients may be required in the formulation of ballan wrasse

348   diets in order to maximise growth and feeding efficiency. Nevertheless, it must be

349   acknowledged that the methodology used (faecal material collected every 3 days over a period

350   of 2 to 4 weeks instead of stripping) in the current study may have resulted in overestimated

351   ADCs, though siphoning was performed regularly on collection days to minimise the risk of

352   leaching from the faecal matter. While the stripping method for collecting faeces may be

353   preferable in this context (Kaushik, 2002) it could not be used given the small size of the fish

354   (juveniles).

355   It is known that fish proximate composition is determined by both endogenous factors

356   (e.g. size, age, metabolic demands) and exogenous factors (e.g. diets, temperature) as described

357   by Shearer (1994). In the present study, the analysis of the macronutrients contained in the diets

358   revealed a higher content of proteins (CP), lipids (CL) and ash in OS2 and a significantly higher

359   level of carbohydrates (CHO) in Symbio. While the diets did not impact significantly on the

360   growth nor the proximate composition of the whole fish, temperature had a clear effect. Indeed,

361   an increase in temperature resulted in lower moisture, ash and carbohydrate as well as higher

362   lipid content. Interestingly, fish protein contents (64.8 – 67.3 %) were below levels reported in

363   wild ballan wrasse (71.1 %) (Hamre et al., 2013). Fish growth and muscular construction

364   requires proteins (Chou et al., 2001; Yang et al., 2002) and therefore, the dietary protein intake

365   is an essential exogenous factor when looking at enhancing growth. It has been shown that

366   increasing the dietary protein level in olive flounder (*Paralichthys olivaceus*) and European

367   sea bass diets from 45 % to 55 % resulted in increased weight gain (+ 16 % in flounder and

368   +23 % in sea bass), with a positive effect on feeding efficiency (Ballestrazzi et al., 1994; Kim

369   et al., 2002). A study by Hamre et al. (2013) showed that the highest growth in ballan wrasse

370   juveniles was obtained in diets containing 65 % CP. In the present experiment, growth may

371   have not been maximal due to a lack of dietary proteins, which were only 51 and 55 % CP in

372   Symbio and OS2, respectively. As for energetic requirements, fish favour (in the following

373   order) lipids, carbohydrates and proteins as energy sources (Kaushik, 2002) and it is known

374   that temperature affects carbohydrate utilisation (Hemre et al., 2002). In the present study, the

375   higher temperature may have resulted in protein sparing with fish using carbohydrates as

376   energy source instead as shown in Atlantic salmon (Hemre et al., 2002). This would explain

377   increased muscular gain and lower carbohydrate levels in fish reared at a higher temperature.

378   In addition, the fact that body carbohydrate levels were significantly reduced in fish at 13 °C

379   compared to 10 °C suggests that carbohydrates are better utilised by the species at higher

380   temperatures, therefore leading to protein sparing. Importantly, the diets tested were close to

381   being isoenergetic (19.5-20.1 MJ kg^{-1}) but OS2 contained more crude lipids. This resulted in a

382   carbohydrate/lipid ratio (CHO/L) of 1.2 in OS2 against 2.6 in Symbio. Studies have shown that

383   a $1 \leq$ CHO/L < 2 resulted in the best growth performances in sea bream (*Sparus latus*) (Hu et

384   al., 2007) and African catfish (*Clarias gariepinus*) (Ali and Jauncey, 2004). Diets with a

385   CHO/L ≥ 2 resulted in reduced growth in those species. The CHO/L ratio of OS2 seems

386   therefore more appropriate according to the above-mentioned studies. Interestingly, the

387   significantly lower DFI observed in fish fed OS2 at 13 and 16 °C and the lack of difference in

388   growth between diets indicates that OS2 was better utilised than Symbio by the fish. Whether

389   this was due to higher protein levels or better CHO/L ratio in OS2 cannot be resolved at this

390   stage and further nutritional studies will be required.

391   HSI data indicated that fish reared at 10 ℃ had a larger liver compared to fish at higher

392   temperatures, suggesting that ballan wrasse juveniles use their liver to stock lipids at low

393   temperature, probably as glycogen, in a similar way to that of Atlantic cod *Gadus morhua*

394   (Jobling, 1988; Dos Santos et al., 1993) and common carp *Cyprinus carpio* (Shikata et al.,

395   1995). The lipid analysis of the liver showed that fatty acid levels were not affected by

396   temperature but were by diet. For instance, livers from fish fed Symbio exhibited increased

397   levels of n-6 PUFA, mostly linoleic acid (18:2n-6), reflecting the fatty acid composition of the

398   Symbio diet, rich in this fatty acid which is the precursor of arachidonic acid (ARA, 20:4n-6).

399   ARA is the precursor of bioactive eicosanoids such as prostaglandins, leukotrienes and lipoxins

400   which actively influence immune function (Rombenso et al., 2016) hence it is known to be an

401   essential fatty acid for most of the marine finfish species (Glencross, 2009). However, the

402   relative higher content of linoleic acid in the livers of fish fed Symbio did not translate into

403   higher levels of ARA, most likely as it appears that ballan wrasse do not have Δ5 desaturase

404   activity (Kabeya et al., 2018). This may imply that in order to reach the ARA requirement for

405   the species, which currently remains unknown, ARA should be supplied to ballan wrasse

406   directly in the diet and not in the form of its metabolic precursor (i.e. linoleic acid) (Bell and

407   Sargent, 2003; Kabeya et al., 2018). In addition, an increase in the lipid content of the livers

408   and a decrease of the whole carcasses' carbohydrate content was observed as the temperature

409   increased. It can therefore be hypothesised that ballan wrasse juveniles utilise carbohydrates as

410   preferential source of energy at high temperature whereas they preferentially use their liver

411   glycogen reserves at low temperature. This is in agreement with studies investigating energy

412   storage and lipids in Atlantic salmon (Ruyter et al., 2006) and European sea bass (Moreira et

413   al., 2008). Mortality during juvenile on growing of ballan wrasse, as for most other marine

414   farmed fish species, is a critical issue which restricts hatchery productivity. While the aetiology

415   of mortality is multifactorial and relates to the overall robustness of the stocks, temperature can

416 be a significant contributing factor by promoting the multiplication of pathogens in the culture

417 system at higher temperatures. In the present study, mortality remained similar across

418 treatments and in line with the rates reported by commercial hatcheries during the nursery

419 stage. It is however worth noting that a subtle increase in mortality (not statistically significant)

420 was observed in fish reared at 16 ℃, however there was no apparent drivers of this. Health

421 management is critical and ballan wrasse hatcheries are facing significant losses mostly due to

422 bacterial infections caused by Atypical *Aeromonas salmonicida*, which is known to be virulent

423 above 16 ℃ (Vågnes et al., 2014; Biering et al., 2016). The *post mortem* analysis of the morts,

424 collected during the trial, by the Institute of Aquaculture's fish veterinarian suggested that

425 *Aeromonas salmonicida* was the main cause of death. Bearing this in mind, a trade-off must be

426 met by which growth is optimised at higher temperatures without compromising health due to

427 opportunistic pathogens.

428   In conclusion, growth and feeding efficiency of ballan wrasse juveniles were

429 significantly improved in fish reared at a constant temperature of 16 ℃ compared to 13 and 10

430 ℃. The data also suggested that further growth enhancement may be obtained at higher

431 temperatures than 16 ℃. Ultimately, rearing fish at 16 ℃ could reduce the production time by

432 more than 4 months in the nursery, with subsequent earlier deployment of ballan wrasse.

433 However, increasing temperature is costly and a full cost-benefit analysis would be required

434 based on shorter time to deployment or increased deployment size, which may lead to improved

435 deployment and better robustness at sea. The macronutrient analysis of the diets and whole

436 body suggested a lack of protein in two of the most commonly used ballan wrasse on-growing

437 diets and a potential issue with the CHO/L ratio in one of them. The digestibility data showed

438 that protein, lipid and energy sources were poorly absorbed by ballan wrasse and attention

439 should be put into identifying easily digestible nutrients for the species. Dietary protein quality

440 and level, dietary CHO/L and nutrient digestibility appear to be critical components for the

18

441   development of suitable diets for this novel species. Overall, this research contributes to the

442   development and optimisation of ballan wrasse aquaculture as an alternative and more

443   sustainable cleaner fish source to wild fisheries.

444

445   **Acknowledgments**

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Table 1. Water quality analyses of the three RAS performed daily during the experiment.

Parameter	RAS 1	RAS 2	RAS 3
NH_4 (mg l^{-1})	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
NO_2 (mg l^{-1})	1.3 ± 1.2	1.0 ± 0.8	0.8 ± 0.8
NO_3 (mg l^{-1})	37.2 ± 48.2	21.9 ± 23.2	18.9 ± 13.7
pH	7.7 ± 0.1	7.7 ± 0.1	7.7 ± 0.1
Salinity (ppt)	34 ± 0	34 ± 0	34 ± 0
Oxygen (%)	95.9 ± 12.1	96.6 ± 8.3	99.5 ± 12.3
Temperature (º C)	15.8 ± 0.3	13.0 ± 0.3	10.6 ± 0.5
Recirculation rate (% day^{-1})	67.9 ± 2.9	69.6 ± 4.9	69.3 ± 4.6

Table 2. Proximate composition and selected fatty acids levels of the commercial on-growing ballan wrasse diets (Otohime S2: OS2; BioMar Symbio: Symbio). Data represents means ± SD (technical duplicate).

Diets	OS2	Symbio
Type	Marumerised	Extruded
Size (mm)	0.92 – 1.8	1.3
Proximate composition (% DW)		
Moisture	6.7 ± 0.0	8.8 ± 0.0
Ash	14.2 ± 0.0	9.5 ± 0.1
Crude protein	54.8 ± 0.0	51.0 ± 0.1
Crude lipid	14.3 ± 0.4	10.9 ± 0.3
Crude fibre	2.2 ± 0.1	2.3 ± 0.1
Carbohydrate[1]	16.7 ± 0.3	28.6 ± 0.3
Gross energy (MJ kg^{-1})	20.1 ± 0.0	19.5 ± 0.3
CHO/L[2]	1.2 ± 0.1	2.6 ± 0.1
Selected fatty acids (% of total FA)		
14:00	6.7 ± 0.2	8.1 ± 0.1
15:00	0.5 ± 0.0	0.4 ± 0.0
16:00	19.5 ± 0.0	22.2 ± 0.1
18:00	3.7 ± 0.1	2.5 ± 0.1
Saturates	30.8 ± 0.0	33.6 ± 0.0
16:1n-9	5.6 ± 0.2	6.1 ± 0.1
16:1n-7	0.4 ± 0.0	0.3 ± 0.0
18:1n-9	12.4 ± 0.1	14.3 ± 0.0
18:1n-7	4.3 ± 0.0	4.9 ± 0.1
20:1n-11	3.0 ± 0.1	1.9 ± 0.1
20:1n-9	2.3 ± 0.1	ND
22:1n-11	4.3 ± 0.1	1.9 ± 0.3
Monounsaturates	33.3 ± 0.3	30.4 ± 0.5
18:2n-6	5.1 ± 0.0	11.4 ± 0.2
18:3n-6	0.1 ± 0.0	0.1 ± 0.0
20:4n-6	0.9 ± 0.0	0.5 ± 0.1
22:5n-6	0.4 ± 0.2	0.3 ± 0.2
Total n-6	6.9 ± 0.2	12.6 ± 0.0
18:3n-3	1.7 ± 0.6	1.9 ± 0.8
18:4n-3	2.4 ± 0.0	1.9 ± 0.0
20:4n-3	0.5 ± 0.0	0.3 ± 0.0
20:5n-3	10.2 ± 0.4	9.7 ± 0.3
22:5n-3	1.0 ± 0.1	0.4 ± 0.0
22:6n-3	11.9 ± 0.0	8.0 ± 0.1
Total n-3	27.9 ± 0.1	22.3 ± 0.5

DW: dry weight; FA: fatty acid; ND: not detected.
OS2: krill meal, fish meal, squid meal, potato starch, wheat flour, fish oil, calcium phosphate, guar gum, betaine, brewer's yeast and liquorice plant.
Symbio: krill meal, fish meal, wheat gluten, mineral premix, vitamin/mineral premix, antioxidant, organic acids.
[1]Carbohydrate = 100 – Ash - Crude protein - Crude lipid.
[2]CHO /L: carbohydrate/lipid ratio.

Table 3. Growth performance indicators and mortality in farmed ballan wrasse juveniles reared during 93 days at three different temperatures (10, 13 and 16 ºC) and fed two commercial diets (Otohime S2: OS2; BioMar Symbio: Symbio). Data are expressed as means ± SD (n = 3). Data were analysed by two-way ANOVA (2 diets x 3 temperatures; * $P<0.05$; ** $P<0.01$).

Temperature (°C)	10		13		16		Significance		
Diet	OS2	Symbio	OS2	Symbio	OS2	Symbio	T	D	TxD
Initial Length (cm)	9.5 ± 0.3	9.7 ± 0.3	9.7 ± 0.2	9.6 ± 0.2	9.5 ± 0.2	9.7 ± 0.2	ns	ns	ns
Final Length (cm)	10.4 ± 0.3	10.5 ± 0.2	11.8 ± 0.2	11.5 ± 0.2	12.3 ± 0.3	12.3 ± 0.3	**	ns	ns
Initial Weight (g)	14.6 ± 0.6	14.5 ± 1.6	14.9 ± 0.5	14.6 ± 0.2	15.1 ± 0.3	14.5 ± 0.5	ns	ns	ns
Final Weight (g)	18.5 ± 0.5	18.1 ± 1.9	25.7 ± 1.5	25.6 ± 1.8	35.4 ± 2.5	33.1 ± 2.0	**	ns	ns
K	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.1	ns	ns	ns
HSI (%)	1.35 ± 0.16	1.39 ± 0.38	0.98 ± 0.15	1.08 ± 0.12	0.64 ± 0.10	0.71 ± 0.08	**	ns	ns
SGR (% day^{-1})	0.2 ± 0.1	0.2 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	**	ns	ns
TGC	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	**	ns	ns
FCR	4.8 ± 1.0	5.1 ± 0.9	2.3 ± 0.1	2.7 ± 0.5	1.4 ± 0.1	1.8 ± 0.3	**	ns	ns
DFI (% day^{-1})	0.9 ± 0.2	1.1 ± 0.0	1.2 ± 0.0	1.4 ± 0.0	1.0 ± 0.1	1.4 ± 0.2	**	**	ns
Final Mortality (%)[1]	4.6 ± 2.1	7.5 ± 1.3	5.1 ± 3.0	7.4 ± 4.1	6.8 ± 4.6	10.1 ± 1.6	ns	ns	-

Initial: start of the growth trial at D0: Final: end of the growth trial at D93; DFI: daily feed intake; FCR: feed conversion ratio; HSI: hepatosomatic index; K: condition factor; SGR: specific growth rate; TGC: thermal growth coefficient; ns: not significant.
[1]Mortality data analysed using the non-parametric tests Kruskal-Wallis and Mann-Whitney U for treatments temperature and diets, respectively.

Table 4. Macronutrient composition of the whole body of ballan wrasse juveniles reared at three different temperatures (10, 13 and 16 ºC) and fed two commercial diets (Otohime S2: OS2; BioMar Symbio: Symbio) compared to wild caught ballan wrasse and apparent digestibility coefficients for fish fed Symbio. Data are expressed as means ± SD (*n* = 3). Data for whole body composition were analysed by two-way ANOVA (2 diets x 3 temperatures; * *P*<0.05; ** *P*<0.01). Data for the digestibility has been analysed by one-way ANOVA (1 diet x 3 temperatures; * *P*<0.05; ** *P*<0.01). Superscripts denote significant differences between treatments.

Temperature (°C)	Wild fish[1]	10		13		16		Significance		
Diet		OS2	Symbio	OS2	Symbio	OS2	Symbio	T	D	TxD
Whole body composition (% DW)										
Moisture	73.7 ± 2.4	78.2 ± 1.4	78.7 ± 1.1	76.2 ± 0.9	76.9 ± 0.7	74.7 ± 0.0	75.0 ± 0.2	**	ns	ns
Ash	-	19.0 ± 0.2	18.5 ± 0.8	17.4 ± 0.1	17.0 ± 0.8	16.9 ± 0.7	15.9 ± 0.6	**	ns	ns
Crude protein	71.1 ± 3.9	67.3 ± 0.3	66.4 ± 1.5	66.3 ± 0.3	67.0 ± 0.8	64.8 ± 0.5	66.5 ± 1.8	ns	ns	ns
Crude lipid	12.8 ± 5.7	7.9 ± 0.8	8.6 ± 0.3	11.7 ± 0.4	11.9 ± 1.1	13.8 ± 1.7	13.1 ± 2.3	**	ns	ns
Carbohydrate[2]	-	5.8 ± 0.8	6.4 ± 0.7	4.7 ± 0.7	4.1 ± 0.2	4.5 ± 0.9	4.5 ± 2.1	*	ns	ns
Digestibility (%)										
$ADC_{Proteins}$	-	-	65.2 ± 6.5	-	52.3 ± 7.7	-	47.3 ± 8.2	ns	-	-
ADC_{Lipids}	-	-	54.0 ± 3.4	-	43.0 ± 4.0	-	42.9 ± 1.0	ns	-	-
ADC_{Energy}	-	-	75.6 ± 1.1	-	52.5 ± 7.7	-	52.5 ± 13.3	ns	-	-

Carbohydrates = 100 – Ash - Crude protein - Crude lipid; ADC: apparent digestibility coefficient; D: diet; DW: dry weight; HSI: hepatosomatic index; ns: not significant; T: temperature.
[1] Hamre, 2013; [2] Calculated by subtraction.

Table 5. Lipid content (%) and selected fatty acid composition (% of total FA) of the liver of ballan wrasse reared at three different (10, 13 and 16 ℃) and fed two commercial diets (Otohime S2: OS2; BioMar Symbio: Symbio). Data expressed as means ± SD ($n = 6$). Data were analysed by two-way ANOVA (2 diets x 3 temperatures, $P<0.05$). Data were pooled per diet as there was no significant effect of the temperature. Superscripts denote significant differences between diets.

Diet	OS2	Symbio
Total lipids (%)	9.5 ± 1.9	9.7 ± 3.5
14:0	6.0 ± 0.6	6.0 ± 0.6
15:0	0.6 ± 0.0	0.5 ± 0.0
16:0	20.1 ± 2.0	21.3 ± 1.9
18:0	4.6 ± 0.3	4.7 ± 0.5
Saturates	31.3 ± 2.1	32.4 ± 2.0
16:1n-9	0.4 ± 0.0[a]	0.5 ± 0.1[b]
16:1n-7	6.8 ± 0.7	7.4 ± 1.2
18:1n-9	17.4 ± 1.0	19.3 ± 1.6
18:1n-7	5.2 ± 0.2	5.5 ± 0.2
20:1n-11	2.8 ± 0.3[b]	0.4 ± 0.2[a]
20:1n-9	2.3 ± 0.2	1.9 ± 0.2
20:1n-7	0.2 ± 0.1	0.1 ± 0.1
22:1n-11	2.9 ± 0.4[b]	1.1 ± 0.2[a]
22:1n-9	0.3 ± 0.1	0.1 ± 0.1
Monosaturates	38.4 ± 2.2	36.2 ± 2.2
18:2n-6	6.0 ± 0.4[a]	11.9 ± 0.8[b]
20:2n-6	0.5 ± 0.1[a]	0.7 ± 0.1[b]
20:4n-6	1.4 ± 0.2	0.8 ± 0.2
Total n-6	7.9 ± 0.4[a]	13.3 ± 0.8[b]
18:3n-3	1.2 ± 0.1	1.0 ± 0.1
18:4n-3	1.3 ± 0.1	1.0 ± 0.2
20:4n-3	0.7 ± 0.0	0.3 ± 0.1
20:5n-3	8.5 ± 0.9	8.2 ± 0.5
22:5n-3	0.8 ± 0.3	0.5 ± 0.1
22:6n-3	9.1 ± 1.5	6.2 ± 0.6
Total n-3	21.6 ± 2.5	17.3 ± 0.8
Total PUFA	30.2 ± 2.6	31.4 ± 1.5

Figure 1. Individual length (g, A), individual weight (cm, B), specific growth rate (SGR, % day^{-1}, C), daily feed intake (DFI, % day^{-1}, D), feed conversion ratio (FCR, E) and hepatosomatic index (HSI, F) calculated in relation to rearing temperature (10, 13 and 16 ºC) and independently from the diets (pooled data within each temperature group) with exception of DFI (effect of both temperature and diet without interaction, two-way ANOVA, $P<0.05$). Data are expressed as means ± SD ($n = 6$) where means have been calculated with the data from the 6 tanks within each temperature group. Dashed lines represent 95 % confidence intervals. Letters indicate statistical differences between temperature groups (two-way ANOVA, $P<0.05$).

Figure 2. WLR and least-square regression line based on individuals reared at different temperatures. **10 ºC**: y = 0.0096x$^{3.1913}$, CI-b = 3.02-3.35, r^2 = 0.94, n = 120, isometric growth relationship with P-value = 0.083; **13 ºC**: y = 0.8349x$^{2.994}$, CI-b = 2.62-3.33, r^2 = 0.8349, n = 120, isometric growth relationship with P-value = 0.881; **16 ºC**: y = 0.0077x$^{3.2781}$, CI-b = 3.13-3.42, r^2 = 0.8651, n = 120, positive allometric growth relationship with P-value = 0.013. Dashed lines represent 95% confidence intervals.

Figure 3. Moisture (%, A), ash (% dry weight, B), lipid (% dry weight, C) and carbohydrate (% dry weight, D) whole body contents at the end of the growth trial (93 days) in relation to rearing temperature (10, 13 and 16 ºC) and independently from the diets (pooled data within each temperature group). Data are expressed as means ± SD ($n = 6$) where means have been calculated with the data from the 6 tanks within each temperature group. Letters indicate statistical differences between temperature groups (two-way ANOVA, $P<0.05$).
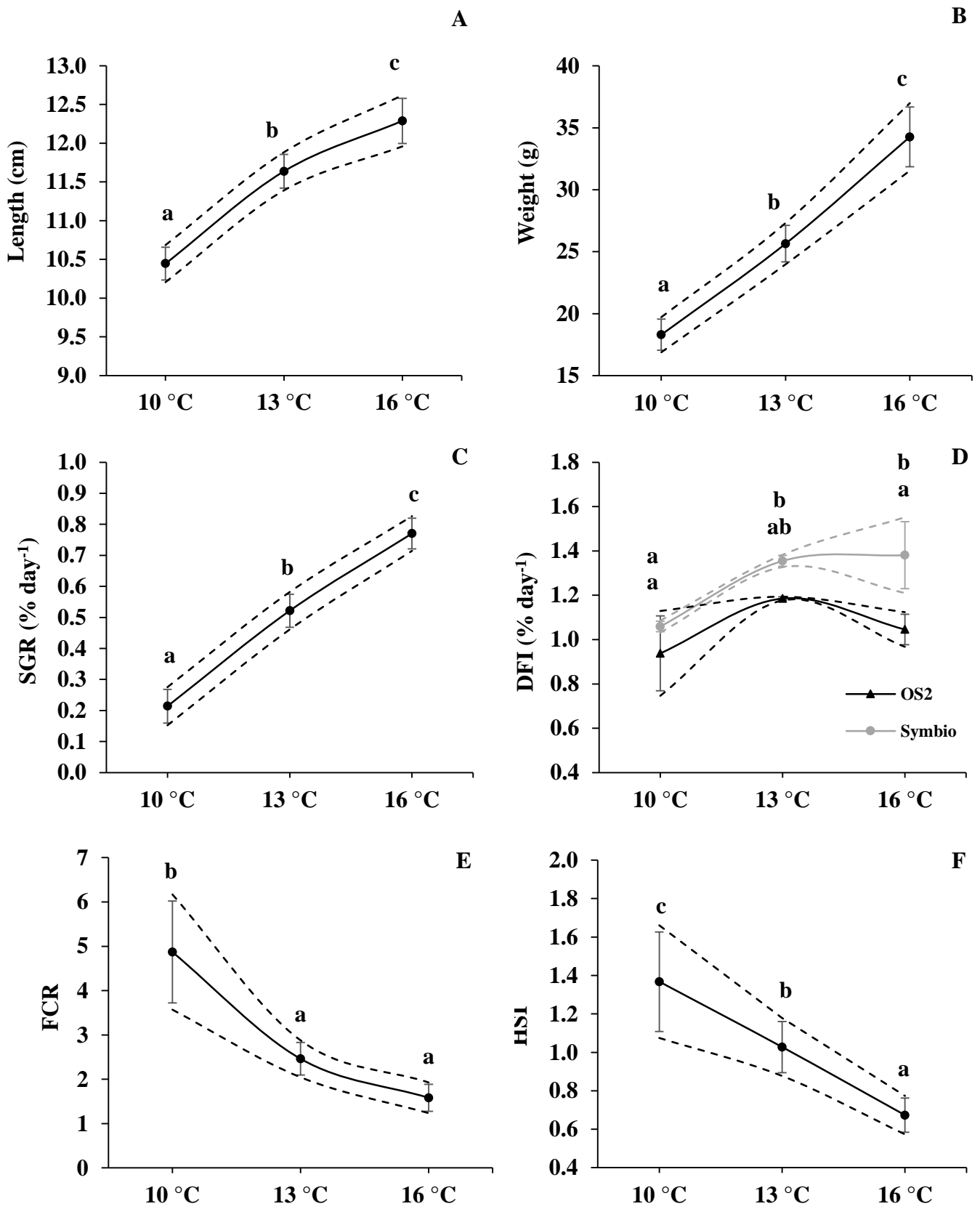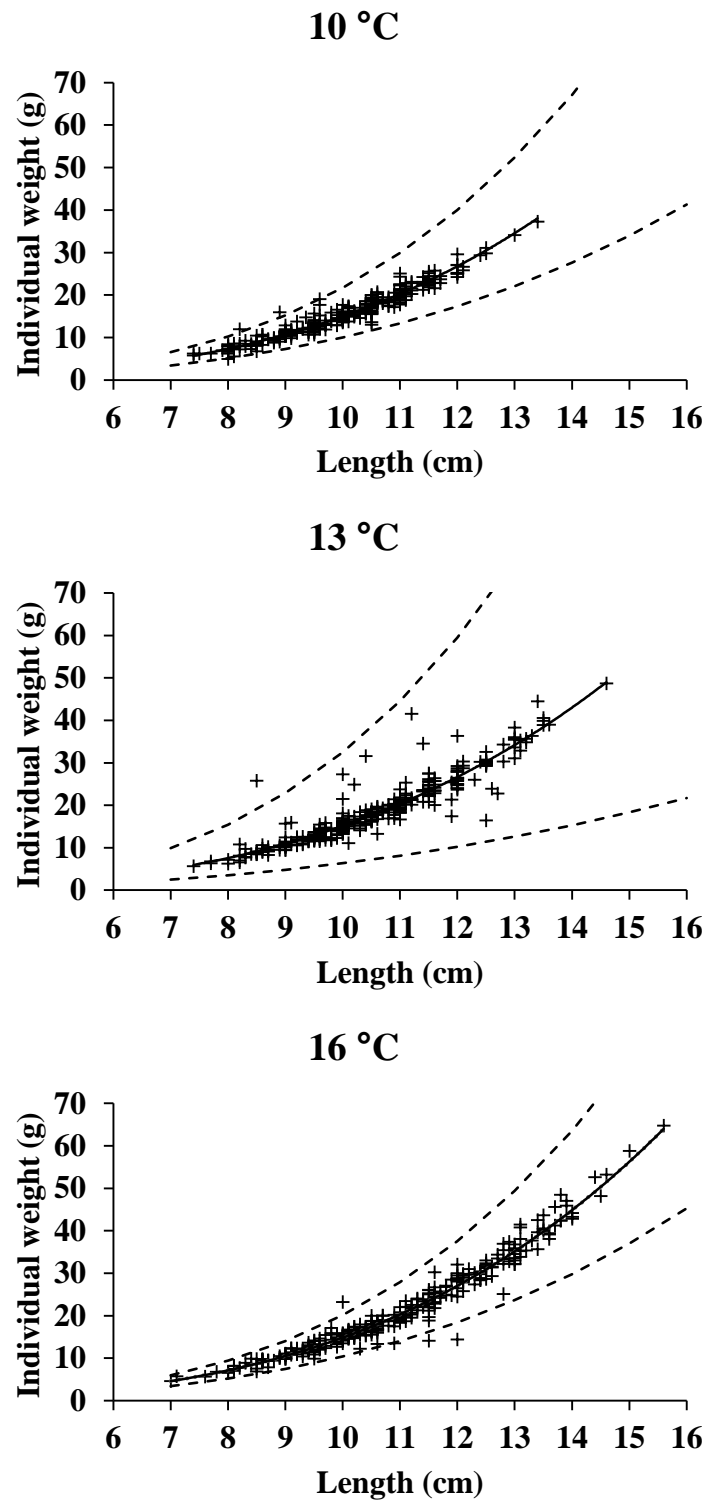
Figure 1.

Figure 2.

10 °C

13 °C

16 °C

Figure 3.