Elevated temperature promotes growth and feed efficiency of farmed ballan wrasse juveniles (*Labrus bergylta*)

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

Addresses

[1] Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK

[2] Instituto de Acuicultura Torre de la Sal, Consejo Superior de Investigaciones Científicas (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain

***Corresponding author**

Prof. Hervé Migaud

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

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

Abstract

The expansion of ballan wrasse farming, used as a biological control against sea lice in Atlantic salmon, is constrained by the slow growth rate in the species and extended period required to reach deployment size. Rearing temperature and diets are the two main growth limiting factors in fish. In this study, farmed ballan wrasse juveniles were reared at 10, 13 and 16 ºC over a period of 3 months and fed two different commercial diets commonly used in marine finfish, Otohime S2 and BioMar Symbio. At the end of the trial, fish growth was +125, +75 and +25 % compared to their initial weight in 16, 13 and 10 ºC treatments, respectively. It was suggested that temperatures above 16 ºC may promote growth even further. Furthermore, feed conversion ratio was significantly improved in fish reared at 16 °C. However, diets did not impact on any of the growth performance indicators although a significantly higher daily feed intake was observed in fish fed BioMar Symbio. Importantly, no significant effects of temperature and diets on mortality and condition factor were observed. No differences were found in the fish (whole-body) macronutrient composition between diets. Analysis of the protein, lipid and energy digestibility revealed lower apparent digestibility coefficients than normally observed in marine species, suggesting the diet formulation is not optimised for the species. Finally, fish reared at 10 ºC showed increased hepatosomatic index, suggesting fat storage in the liver under cold temperatures. These results showed that the production cycle could be shortened by more than 4 months in fish reared at 16 ºC. This could contribute to increase hatchery productivity and meet demand from the salmon production sector while reducing costs associated with the nursery phase although maintaining a constant high temperature would increase operational costs.

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

health and welfare of the fish. Temperature requirements are known for many marine species, which results in the establishment of validated commercial rearing protocols e.g. turbot (*Scophthalmus maximus*) (Burel et al., 1996, Imsland et al., 1996) and European sea bass (*Dicentrachus labrax*) (Person-Le Ruyet et al., 2004). The implementation of temperature optima in the hatcheries subsequently optimises growth and feed efficiency, which ultimately has significant positive impact on production efficiency. In turbot, rearing fish at 17 ºC compared to 8 ºC resulted in a 50 % increase in specific growth rate (SGR) (Burel et al., 1996). In seabass, rearing fish at 22 ºC compared to 16 ºC increased SGR by 75 % and enhanced feed efficiency (Person-Le Ruyet et al., 2004). However, to date, no published data is available on optimal rearing temperature for ballan wrasse and consequently no standardised rearing temperature has been applied in commercial hatcheries. Ballan wrasse is naturally distributed in an area covering the North East Atlantic Ocean (Sayer et al., 1996), where temperature ranges (on average) from 2.5 ºC in winter at the most northern point of their natural distribution (e.g. Norwegian coast) up to 22 ºC in summer in the most Southern regions (e.g. Portuguese coast) (US Department, 1998). Although this indicates a broad temperature tolerance range, species preferences are greatly dependent upon the geographical origin of a specific population as demonstrated by Björnsson et al., (2007) in Atlantic cod (*Gadus morhua*). Thus, in the context of ballan wrasse, production focus must be directed towards optimising environmental conditions for stocks derived from the main production areas.

There is equally limited published data on the nutritional requirements of ballan wrasse. In their natural habitat, they feed on decapods and bivalves as well as smaller amounts of algae and gastropods (Deady and Fives, 1995). However, while their natural diet is not piscivorous, ballan wrasse hatcheries use commercially available diets which are rich in fish meal (Hamre et al., 2013; Kousoulaki et al., 2015). Such diets may not be the most suitable for ballan wrasse. Furthermore, a recent study suggested that ballan wrasse prefer easily digestible ingredients

(e.g. krill or fish protein hydrolysate) as opposed to fish meal diets (Lie et al., 2018). At the moment, there is no data on nutrient digestibility in ballan wrasse, which severely limits the formulation of suitable diets for the species and the associated benefits on feed efficiency.

This study aimed to investigate the effects of temperature on ballan wrasse growth, feed efficiency and nutrient digestibility in relation to diet. To do so, fish were reared under three constant temperatures, within the typical range experienced by cultured ballan wrasse populations and fed with two different commercially available diets formulated for marine finfish species commonly used in ballan wrasse hatcheries.

Materials and Methods

Experimental system and culture conditions

The trial was carried out at the Machrihanish Marine Environmental Research Laboratory (Machrihanish, UK). The main water supply used in the experimental system was filtered to 10 µm through a combination of sand and pressure filters followed by UV treatment (1.18 MJ h^{-1}). The study was performed in 18 circular flat-bottom tanks (100-l) divided evenly between three independent recirculation systems (TMC system 1,000: Tropical Marine Centre, UK) with a water turn over in the tanks of approximately 1 hour. Tanks were illuminated 24 hours with an average intensity of 0.8 W m^{-2} at the water surface. Each tank was stocked with 60 juvenile fish (14.7 ± 0.7 g) produced by the neighbouring commercial ballan wrasse hatchery (MOWI Scotland Ltd, Machrihanish, UK) where they had previously been reared at 13 °C. Fish were acclimated to the study temperatures at a rate of 0.5 ºC day^{-1} and were then maintained at the following experimental temperatures (Table 1). Water quality (e.g. NH_4, NO_2, NO_3, pH, salinity and O_2) was monitored daily in each RAS and no differences were observed between systems (Table 1). At the beginning of the experiment, the stocking density 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 ±

0.8 kg m^{-3} and 16.5 ± 1.6 kg m^{-3} at 10, 13 and 16 ºC, respectively, which remains within commercial stocking density ranges.

Feeding and digestibility study

For the first 93 days, termed the "growth" trial, the fish were fed two commercial marine diets, namely Otohime S2 (Marubeni Nissin Feed Co., Tokyo, Japan) and BioMar Symbio (BioMar, Brande, Denmark), which are commonly used for on-growing in commercial ballan wrasse hatcheries in the UK and Norway. The biochemical analysis (Table 2) showed that the diets were close to isoenergetic (20.1 ± 0.0 and 19.5 ± 0.3 MJ kg^{-1} for OS2 and Symbio, respectively). OS2 appeared to have a lower moisture content (6.7 ± 0.0 %) compared to Symbio (8.8 ± 0.0 %). Ash, crude protein (CP) and crude lipid (CL) were higher in OS2 (14.2 ± 0.0 %, 54.8 ± 0.0 % and 14.3 ± 0.4 %, respectively) compared to Symbio (9.5 ± 0.1 %, 51.0 ± 0.1 % and 10.9 ± 0.3 %, respectively). The carbohydrate content of Symbio was higher than in OS2 (28.6 ± 0.3 % and 16.7 ± 0.3 %, respectively). Finally, the carbohydrate/crude lipid ratio (CHO/L) was more than two times higher in Symbio than OS2 (2.6 ± 0.1 and 1.2 ± 0.1, respectively). The diets were tested in triplicate (n = 3) within each independent recirculation system. Feeds were automatically distributed using Eheim twin-screw feeders controlled by a central command unit. Throughout the experiment, all fish were fed to satiation plus excess confirmed by the presence of a quantifiable number of uneaten pellets at the bottom of the tanks. Feed recovery was done daily by siphoning the bottom of the tanks to collect uneaten pellets which were weighed and converted into a dry weight using previously made standard curves. At the end of the 93 days, the trial was extended for another 4 weeks to collect enough faeces for digestibility analyses. Yttrium oxide, an inert digestibility marker, was added to BioMar Symbio during the aquafeed production process at approximately 350 mg kg^{-1}. For technical reasons, yttrium could not be added to OS2 and therefore, digestibility data could

only be obtained from Symbio fed fish. Fish were fed Symbio continuously to satiation for 2 days before turning off the feeders and flushing the tanks to remove the faeces and uneaten food. On the 3rd day, the freshly produced faeces were collected by siphoning every 2 hours, and storing samples frozen at -20 ºC. The procedure was repeated until enough faecal material was collected for nutritional analysis (*circa* 28 g of wet faeces per tank).

Sampling

The sampling regime during the growth trial included an initial (D0), interim (D45) and final (D93) sample points for which the biomass (g) and the total number of fish per tank were recorded. Also, the total length (cm) and weight (g) of 20 fish per tank were recorded and allowed the calculation of K factors using the following equation:

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

Individual weight (g), feed conversion ratio (FCR), specific growth rate (SGR), thermal growth coefficient (TGC) and daily feed intake (DFI) were calculated as follow using the biomass data:

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

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

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

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

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

At the end of the growth trial, six fish per tank were sacrificed, pooled (two pools of three fish per tank) and preserved at -20 °C for proximate composition analysis. Another six fish were sacrificed, and their livers weighed to calculate the hepatosomatic index ($\text{HSI} = 100 \times (\text{weight liver, g fish}^{-1}) / (\text{body weight, g fish}^{-1})$). The livers were then pooled (two pools of three livers per tank) and preserved at -20 ºC for lipid composition analysis.

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

holding that temperature). Digests were transferred into a volumetric flask and made up into x 25 dilutions with distilled water. Samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Scientific Model X Series 2, USA). Apparent digestibility coefficient (ADC) was calculated as follow:

$ADC_{nutrient}$ (%) = [1 – ($Nutrient_{faeces}$, %) / ($Nutrient_{diet}$, %) x ($Yttrium_{faeces}$, mg kg^{-1}) / ($Yttrium_{diet}$, mg kg^{-1})] x 100

Statistics

All data are presented as mean ± standard deviation (SD). Percentage data were subjected to arcsine square-root transformation prior to statistical analyses. Normality and homogeneity of variance in the data were confirmed using Shapiro-Wilk and Levene's tests, respectively. Growth indicators and nutritional data were analysed by two-way ANOVA using "Temperature" and "Diet" as factors, followed by Tukey's post-hoc test when relevant. Mortality data was not normally distributed and was therefore analysed using the non-parametric tests Kruskal-Wallis and Mann-Whitney U for treatments temperature and diets, respectively. Apparent digestibility for fish fed Symbio was analysed by one-way ANOVA followed by Tukey's post-hoc test. All treatment effects were considered significant at a significance level of $P<0.05$. To test for isometry in growth rate the weight-length relationship (WLR): $W = aL^b$, where W is the body weight, L the standard length and a and b are parameters of the relationship was transformed into its logarithmic equivalent: log(BW) = log(a) + b*log(FL) for analysis by least-square regression based (Froese, 2006). Significant variations from the isometry (slope = 3) were determined using a Student *t*-test ($\alpha = 0.05$) (Arslan et al., 2004). All data were analysed using SPSS (IBM SPSS Statistics 23, NY, USA) and Microsoft Excel (v16, WA, USA).

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

13 ºC and remained elevated at 16 ºC. Overall, DFI was significantly higher at 16 ºC in fish fed Symbio compared to those fed OS2 (Fig. 1D).

There was no impact of diet within a given temperature on observed FCR. Significant differences in FCR were observed in relation to temperature with reduced FCR in fish reared at 16 ºC (1.4 ± 0.1 and 1.8 ± 0.3 for fish fed OS2 and Symbio, respectively) and 13 ºC (2.3 ± 0.1 and 2.7 ± 0.5 for fish fed OS2 and Symbio, respectively) compared to 10 ºC (4.8 ± 1.0 and 5.1 ± 0.9 for fish fed OS2 and Symbio, respectively) (Table 3, Fig. 1E).

No differences in K were found between treatments (Table 3). Mortality rates were not significantly affected by treatments, ranging from 4.6 ± 2.1 to 10.1 ± 1.6 % at the end of the experiment (Table 3). Hepatosomatic index (HSI) was significantly higher in fish reared at 10 ºC (1.35 ± 0.16 and 1.39 ± 0.38 % for OS2 and Symbio, respectively) than at 16 ºC (0.64 ± 0.12 and 0.71 ± 0.08 % for OS2 and Symbio, respectively) (Table 3, Fig. 1F).

Proximate analyses

Moisture and ash contents were significantly higher in fish reared at 10 ºC compared to 16 ºC (Table 4, Fig. 3A and 3B). Crude protein content in fish was not affected by any treatments but appeared to be lower than reported in wild ballan wrasse (mean of 66.4 ± 1.2 and 71.1 ± 3.9 %, respectively) (Table 4). Crude lipid levels were significantly higher in fish reared at 16 ºC (13.8 ± 1.7 and 13.1 ± 2.3 % for OS2 and Symbio, respectively) compared to 10 ºC (7.9 ± 0.8 and 8.6 ± 0.3 % for OS2 and Symbio, respectively) (Fig. 3C). CL levels reported in wild fish (12.8 ± 5.7 %) appeared to be comparable to that observed in 13 ºC and 16 °C treatments. Carbohydrates were significantly lower in fish reared at 13 ºC (4.7 ± 0.7 and 4.1 ± 0.2 % for OS2 and Symbio, respectively) compared to 10 ºC (5.8 ± 0.8 and 6.4 ± 0.7 % for OS2 and Symbio, respectively) (Fig. 3D). Carbohydrates appeared to be lower at 16 ºC (4.5 ± 0.9 and

4.5 ± 2.1 % for OS2 and Symbio, respectively) compared to 10 °C, but not significantly (Fig. 3D). No carbohydrate data is available for wild ballan wrasse.

In terms of macronutrient uptake, digestibility analysis could be done in Symbio due to the inclusion of yttrium oxide in this diet. Overall, ADC levels were low (i.e. <76 %) (Table 4). The increase of the rearing temperature from 10 ºC to 13 ºC resulted in an apparent reduction in digestibility coefficient of proteins ($ADC_{Proteins}$), lipids (ADC_{Lipids}) and energy (ADC_{Energy}) although this was not significant at the $P<0.05$ level (one-way ANOVA, $P = 0.063$, $P = 0.134$ and $P = 0.084$ for $ADC_{Proteins}$, ADC_{Lipids} and ADC_{Energy}, respectively).

The lipid content of the liver remained constant between treatments (mean of 9.6 ± 2.8 %) although a decreasing trend at lower temperature was observed (Table 5). Liver of fish fed Symbio displayed a higher content of linoleic acid (18:2n-6) (~+97 % across temperature treatments) and n-6 PUFA (~+68 % across temperature treatments) (Table 5).

Discussion

The salmon industry is currently seeking to increase the number of farmed deployed ballan wrasse to tackle sea lice outbreaks and reduce pressure on wild stocks. However, the production of this new aquaculture species is hampered by its slow growth, which is attributed to intrinsic characteristics of the fish but also to the lack of understanding of the species environmental and nutritional preferences. As reported in many temperate marine fish species, an increase in the rearing temperature can promote growth until it reaches a plateau before then rapidly declining (Jobling, 1996). Wild ballan wrasse can live in a wide range of temperatures as experienced in their natural habitats (e.g. 6 to 17 ºC in Scotland, Marine Scotland, 2011) and no standardisation between commercial hatcheries have been applied yet with water temperature during juvenile rearing ranging from 8 to 16 °C (Featherstone, P.; Barge, A.; personal communication). Results from the present study clearly showed enhanced growth

performance (i.e. weight gain, SGR, TGC) of ballan wrasse juveniles reared at a constant temperature of 16 °C. Ideally, the study should have normalised sampling times with degree days. However, this would have extended the study by 56 days and led to stocking densities in the 16 °C treatment exceeding accepted limits. Therefore, it was decided to limit the study to a fixed number of days with a minimum of doubling of fish weight in the upper temperature treatment.

The Weight-Length Relationship (WLR) analysis showed that fish reared at 16 ºC displayed a positive allometric growth as reported previously in a mixed-gender population of wild ballan wrasse exposed to similar natural temperatures (Leclercq et al., 2014b). However, under hatchery conditions, growth pattern appeared to change to isometric in fish reared at 10 and 13 ºC. It is known that for teleosts such as Atlantic salmon, heavier individuals tend to exhibit a positive allometric relationship often resulting from a higher condition factor K (Leclercq et al., 2010). Although in the present study no differences in K factors were observed across treatments, data showed that ballan wrasse somatic growth and subsequent weight gain was enhanced at 16 °C compared to that of lower temperatures, resulting in heavier fish hence a positive allometric growth. In production, farmers should closely monitor the condition of their fish, particularly when reared at 16 °C, so any change in fish condition (i.e. increase of K above normal values) can be detected and mitigated if possible, through nutritional interventions.

A significant difference in fish length between rearing temperatures was also observed at the end of our experiment suggesting that higher temperatures promote somatic growth as well as skeleton development (Schneider et al., 2000). Condition factor was not affected by either treatment and remained within normal levels for the species according to Skiftesvik et al. (2013) and Leclercq et al. (2014a). In those studies, K of ballan wrasse juveniles coming from a hatchery environment was higher (≥1.5) than for wild fish or fish that had been deployed for 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 ºC. While DFI did display a differential response in relation to diet,
322 in both cases DFI reached a plateau after 13 ºC (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 ºC, 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

% and 52.5-75.6 %, respectively). These results support the fact that available diets used for ballan wrasse may not be fully suitable for the species. A study from Kousoulaki et al. (2015) showed that the inclusion of shrimp and krill hydrolysate, both very high-quality protein sources, in ballan wrasse weaning diets had a positive effect on the growth and survival of the fish compared to diets containing commercial medium quality fish meal, similarly to that of the present study. Although Kousoulaki's study lacked data on digestibility, it suggests that alternative and high-quality ingredients may be required in the formulation of ballan wrasse diets in order to maximise growth and feeding efficiency. Nevertheless, it must be acknowledged that the methodology used (faecal material collected every 3 days over a period of 2 to 4 weeks instead of stripping) in the current study may have resulted in overestimated ADCs, though siphoning was performed regularly on collection days to minimise the risk of leaching from the faecal matter. While the stripping method for collecting faeces may be preferable in this context (Kaushik, 2002) it could not be used given the small size of the fish (juveniles).

It is known that fish proximate composition is determined by both endogenous factors (e.g. size, age, metabolic demands) and exogenous factors (e.g. diets, temperature) as described by Shearer (1994). In the present study, the analysis of the macronutrients contained in the diets revealed a higher content of proteins (CP), lipids (CL) and ash in OS2 and a significantly higher level of carbohydrates (CHO) in Symbio. While the diets did not impact significantly on the growth nor the proximate composition of the whole fish, temperature had a clear effect. Indeed, an increase in temperature resulted in lower moisture, ash and carbohydrate as well as higher lipid content. Interestingly, fish protein contents (64.8 – 67.3 %) were below levels reported in wild ballan wrasse (71.1 %) (Hamre et al., 2013). Fish growth and muscular construction requires proteins (Chou et al., 2001; Yang et al., 2002) and therefore, the dietary protein intake 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 $CHO/L \geq 2$ 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.

HSI data indicated that fish reared at 10 ºC had a larger liver compared to fish at higher temperatures, suggesting that ballan wrasse juveniles use their liver to stock lipids at low temperature, probably as glycogen, in a similar way to that of Atlantic cod *Gadus morhua* (Jobling, 1988; Dos Santos et al., 1993) and common carp *Cyprinus carpio* (Shikata et al., 1995). The lipid analysis of the liver showed that fatty acid levels were not affected by temperature but were by diet. For instance, livers from fish fed Symbio exhibited increased levels of n-6 PUFA, mostly linoleic acid (18:2n-6), reflecting the fatty acid composition of the Symbio diet, rich in this fatty acid which is the precursor of arachidonic acid (ARA, 20:4n-6). ARA is the precursor of bioactive eicosanoids such as prostaglandins, leukotrienes and lipoxins which actively influence immune function (Rombenso et al., 2016) hence it is known to be an essential fatty acid for most of the marine finfish species (Glencross, 2009). However, the relative higher content of linoleic acid in the livers of fish fed Symbio did not translate into higher levels of ARA, most likely as it appears that ballan wrasse do not have Δ5 desaturase activity (Kabeya et al., 2018). This may imply that in order to reach the ARA requirement for the species, which currently remains unknown, ARA should be supplied to ballan wrasse directly in the diet and not in the form of its metabolic precursor (i.e. linoleic acid) (Bell and Sargent, 2003; Kabeya et al., 2018). In addition, an increase in the lipid content of the livers and a decrease of the whole carcasses' carbohydrate content was observed as the temperature increased. It can therefore be hypothesised that ballan wrasse juveniles utilise carbohydrates as preferential source of energy at high temperature whereas they preferentially use their liver glycogen reserves at low temperature. This is in agreement with studies investigating energy storage and lipids in Atlantic salmon (Ruyter et al., 2006) and European sea bass (Moreira et al., 2008). Mortality during juvenile on growing of ballan wrasse, as for most other marine farmed fish species, is a critical issue which restricts hatchery productivity. While the aetiology of mortality is multifactorial and relates to the overall robustness of the stocks, temperature can

be a significant contributing factor by promoting the multiplication of pathogens in the culture system at higher temperatures. In the present study, mortality remained similar across treatments and in line with the rates reported by commercial hatcheries during the nursery stage. It is however worth noting that a subtle increase in mortality (not statistically significant) was observed in fish reared at 16 ºC, however there was no apparent drivers of this. Health management is critical and ballan wrasse hatcheries are facing significant losses mostly due to bacterial infections caused by Atypical *Aeromonas salmonicida*, which is known to be virulent above 16 ºC (Vågnes et al., 2014; Biering et al., 2016). The *post mortem* analysis of the morts, collected during the trial, by the Institute of Aquaculture's fish veterinarian suggested that *Aeromonas salmonicida* was the main cause of death. Bearing this in mind, a trade-off must be met by which growth is optimised at higher temperatures without compromising health due to opportunistic pathogens.

In conclusion, growth and feeding efficiency of ballan wrasse juveniles were significantly improved in fish reared at a constant temperature of 16 ºC compared to 13 and 10 ºC. The data also suggested that further growth enhancement may be obtained at higher temperatures than 16 ºC. Ultimately, rearing fish at 16 ºC could reduce the production time by more than 4 months in the nursery, with subsequent earlier deployment of ballan wrasse. However, increasing temperature is costly and a full cost-benefit analysis would be required based on shorter time to deployment or increased deployment size, which may lead to improved deployment and better robustness at sea. The macronutrient analysis of the diets and whole body suggested a lack of protein in two of the most commonly used ballan wrasse on-growing diets and a potential issue with the CHO/L ratio in one of them. The digestibility data showed that protein, lipid and energy sources were poorly absorbed by ballan wrasse and attention should be put into identifying easily digestible nutrients for the species. Dietary protein quality and level, dietary CHO/L and nutrient digestibility appear to be critical components for the

development of suitable diets for this novel species. Overall, this research contributes to the development and optimisation of ballan wrasse aquaculture as an alternative and more sustainable cleaner fish source to wild fisheries.

Acknowledgments

The project and PhD studentship TCR were supported by a project co-funded by the Scottish Aquaculture Innovation Centre (SAIC) and industry partners. The authors are grateful to the staff at the Marine Environmental Research Laboratory (Machrihanish, UK) for their help with the daily husbandry, MOWI Scotland Ltd and Scottish Sea Farms Ltd for the provision of fish and to BioMar UK (especially Dr. Daniel Leeming) for providing the Symbio diet and guidance for the trial design.

References

Ali, M.Z., Jauncey, K., 2004. Optimal dietary carbohydrate to lipid ratio in African catfish *Clarias gariepinus* (Burchell 1822). Aquac. Int. 12, 169–180.

AOAC, 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC.

Arslan, M., Yildirim, A., Bektas, S., 2004. Length-weight relationship of brown trout, *Salmo trutta* L., inhabiting Kan Stream, Coruh Basin, north-eastern Turkey. Turkish J. Fish. Aquat. Sci. 4, 45–48.

Ballestrazzi, R., Lanari, D., D'Agaro, E., Mion, A., 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*). Aquaculture 127, 197–206.

Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218, 491–499.

Biering, E., Vaagnes, Krossøy, B., Gulla, S., Colquhoun, D.J., 2016. Challenge models for atypical *Aeromonas salmonicida* and *Vibrio anguillarum* in farmed Ballan wrasse (*Labrus bergylta*) and preliminary testing of a trial vaccine against atypical *Aeromonas salmonicida*. J. Fish Dis. 39, 1257–1261.

Björnsson, B., Steinarsson, A., Árnason, T., 2007. Growth model for Atlantic cod (*Gadus morhua*): Effects of temperature and body weight on growth rate. Aquaculture 271, 216–226.

Blanco Gonzalez, E., de Boer, F., 2017. The development of the Norwegian wrasse fishery and the use of wrasses as cleaner fish in the salmon aquaculture industry. Fish. Sci. 83, 661-670.

Bolton-Warberg, M., 2017. An overview of cleaner fish use in Ireland. J. Fish Dis. 41, 935-939.

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. Fish Physiol. 8, 279–352.

Brooker, A.J., Skern-Mauritzen, R., Bron, J.E., 2018a. Production, mortality, and infectivity of planktonic larval sea lice, *Lepeophtheirus salmonis* (Kroyer, 1837): Current knowledge and implications for epidemiological modelling. ICES J. Mar. Sci. 75, 1214–1234.

Brooker, A.J., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A., Migaud, H., 2018b. Sustainable production and use of cleaner fish for the biological control of sea lice : recent advances and current challenges. Vet. Rec 183, 383.

Burel, C., Person-Le Ruyet, J., Gaumet, F., Le Roux, A., Sévère, A., Boeuf, G., 1996. Effects of temperature on growth and metabolism in juvenile turbot. J. Fish Biol. 49, 678–692.

Cho, S.H., Lee, S.M., Lee, S.M., Lee, J.H., 2005. Effect of dietary protein and lipid levels on growth and body composition of juvenile turbot (*Scophthalmus maximus* L) reared under optimum salinity and temperature conditions. Aquac. Nutr. 11, 235–240.

Chou, R.L., Su, M. Sen, Chen, H.Y., 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 193, 81–89.

Christie, W.W., 2003. Isolation, Separation, identification and lipidomic analysis, The Oily Press, Bridgwater, UK. Oily Press Imprint PJ Barnes Assoc. 1-289 ISBN:978-0953194957.

Deady, S., Fives, J.M., 1995. Diet of ballan wrasse, *Labrus bergylta*, and some comparisons with the diet of corkwing wrasse, *Crenilabrus melops*. J. Mar. Biol. Assoc. United Kingdom 75, 651–665.

Dos Santos, J., Burkow, I.C., Jobling, M., 1993. Patterns of growth and lipid deposition in cod (*Gadus morhua* L) fed natural prey and fish based feeds. Aquaculture 110, 173–189.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem 226, 497–509.

501 Froese, R., 2006. Cube law, condition factor and weight-length relationships: History, meta-
502 analysis and recommendations. J. Appl. Ichthyol. 22, 241–253.
503 Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture
504 species. Rev. Aquac. 1, 71–124.
505 Glencross, B.D., Booth, M., Allan, G.L., 2007. A feed is only as good as its ingredients - A
506 review of ingredient evaluation strategies for aquaculture feeds. Aquac. Nutr. 13, 17–34.
507 Hamre, K., Nordgreen, A., Grøtan, E., Breck, O., 2013. A holistic approach to development of
508 diets for Ballan wrasse (*Labrus bergylta*) – a new species in aquaculture. PeerJ 1, e99.
509 Helland, S., Dahle, S.W., Hough, C., Borthen, J., 2013. Production of ballan wrasse (*Labrus*
510 *bergylta*) - Science and practice, Report. Oslo.
511 Hemre, G.I., Mommsen, T.P., Krogdahl, Å., 2002. Carbohydrates in fish nutrition: effects on
512 growth, glucose metabolism and hepatic enzymes. Aquac. Nutr. 8, 175–194.
513 Hu, Y.H., Liu, Y.J., Tian, L.X., Yang, H.J., Liang, G.Y., Gao, W., 2007. Optimal dietary
514 carbohydrate to lipid ratio for juvenile yellowfin seabream (*Sparus latus*). Aquac. Nutr.
515 13, 291–297.
516 Imsland, A.K., Sunde, L.M., Folkvord, A., Stefansson, S.O., 1996. The interaction of
517 temperature and fish size on growth of juvenile turbot. J. Fish Biol. 49, 926–940.
518 Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Foss, A., Vikingstad, E., Elvegård,
519 T.A., 2014. The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice
520 (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon
521 (*Salmo salar* L.). Aquaculture 424–425, 18–23.
522 Jobling, M., 1996. Temperature and growth: modulation of growth rate via temperature change,
523 in: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for Freshwater
524 and Marine Fish. pp. 225–253.

525 Jobling, M., 1988. A review of the physiological and nutritional energetics of cod, *Gadus*
526 *morhua* L., with particular reference to growth under farmed conditions. Aquaculture 70,
527 1-19.
528 Jobling, M., 1981. The influences of feeding on the metabolic rate of fishes: a short review. J.
529 Fish Biol. 18, 385–400.
530 Kabeya, N., Yevzelman, S., Oboh, A., Tocher, D.R., Monroig, O., 2018. Essential fatty acid
531 metabolism and requirements of the cleaner fish, ballan wrasse *Labrus bergylta*:
532 Defining pathways of long-chain polyunsaturated fatty acid biosynthesis. Aquaculture
533 488, 199–206.
534 Kaushik, S.J., 2002. Bioenergetics. In: *Fish Nutrition*, Halver, J.E., Hardy, R.W. (eds). Third
535 edition. Academic Press: San Diego, 1-59.
536 Kaushik, S.J., Covès, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by
537 plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus*
538 *labrax*. Aquaculture 230, 391–404.
539 Kim, K.W., Wang, X.J., Bai, S.C., 2002. Optimum dietary protein level for maximum growth
540 of juvenile olive flounder *Paralichthys olivaceus* (Temminck et Schlegel). Aquac. Res.
541 33, 673–679.
542 Kousoulaki, K., Bogevik, A.S., Skiftesvik, A.B., Jensen, P.A., Opstad, I., 2015. Marine raw
543 material choice, quality and weaning performance of Ballan wrasse (*Labrus bergylta*)
544 larvae. Aquac. Nutr. 21, 644–654.
545 Leclercq, E., Taylor, J.F., Hunter, D., Migaud, H., 2010. Body size dimorphism of sea-reared
546 Atlantic salmon (*Salmo salar* L.): Implications for the management of sexual maturation
547 and harvest quality. Aquaculture 301, 47–56.

548 Leclercq, E., Davie, A., Migaud, H., 2014a. Delousing efficiency of farmed ballan wrasse
549 (*Labrus bergylta*) against *Lepeophtheirus salmonis* infecting Atlantic salmon (*Salmo*
550 *salar*) post-smolts. Pest Manag. Sci. 70, 1274–1282.
551 Leclercq, E., Grant, B., Davie, A., Migaud, H., 2014b. Gender distribution, sexual size
552 dimorphism and morphometric sexing in ballan wrasse *Labrus bergylta*. J. Fish Biol. 84,
553 1842–1862.
554 Lie, K.K., Tørresen, O.K., Solbakken, M.H., Rønnestad, I., Tooming-Klunderud, A.,
555 Nederbragt, A.J., Jentoft, S., Sæle, Ø., 2018. Loss of stomach, loss of appetite?
556 Sequencing of the ballan wrasse (*Labrus bergylta*) genome and intestinal transcriptomic
557 profiling illuminate the evolution of loss of stomach function in fish. BMC Genomics 19,
558 186.
559 Marine Scotland, 2011. Scotland´s Marine Atlas, History. Scottish Government.
560 Moreira, I.S., Peres, H., Couto, A., Enes, P., Oliva-Teles, A., 2008. Temperature and dietary
561 carbohydrate level effects on performance and metabolic utilisation of diets in European
562 sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 274, 153–160.
563 Mundheim, H., Aksnes, A., Hope, B., 2004. Growth, feed efficiency and digestibility in salmon
564 (*Salmo salar* L.) fed different dietary proportions of vegetable protein sources in
565 combination with two fish meal qualities. Aquaculture 237, 315–331.
566 Person-Le Ruyet, J., Mahé, K., Le Bayon, N., Le Delliou, H., 2004. Effects of temperature on
567 growth and metabolism in a Mediterranean population of European sea bass,
568 *Dicentrarchus labrax*. Aquaculture 237, 269–280.
569 Rombenso, A.N., Trushenski, J.T., Jirsa, D., Drawbridge, M., 2016. Docosahexaenoic acid
570 (DHA) and arachidonic acid (ARA) are essential to meet LC-PUFA requirements of
571 juvenile California Yellowtail (*Seriola dorsalis*). Aquaculture 463, 123–134.

572 Ruyter, B., Moya-Falcon, C., Rosenlund, G., Vegusdal, A., 2006. Fat content and morphology
573 of liver and intestine of Atlantic salmon (*Salmo salar*): Effects of temperature and dietary
574 soybean oil. Aquaculture 252, 441–452.
575 Sayer, M.D.J., Treasurer, J.W. and Costello, M.J., 1996, North European wrasse: identification,
576 distribution and habitat, in Wrasse: Biology and Use in Aquaculture, edited by Sayer,
577 M.D.J., Treasurer, J.W. and Costello, M.J.. Fishing News Books Ltd, Oxford, UK, 3–13.
578 Schneider, J.C., Laarman, P.W., and Gowing, H. 2000. Chapter 17: Length-weight
579 relationship, in Manual of fisheries survey methods II: with periodic updates, edited
580 by J.C. Schneider and C. James. Michigan Department of Natural Resources (MDNR),
581 Fisheries Special Report 25, Ann Arbor, Mich. pp. 1–16.
582 Shearer, K.D., 1994. Factors affecting the proximate composition of cultured fishes with
583 emphasis on salmonids. Aquaculture 119, 63–88.
584 Shikata, T., Iwanaga, S., Shimeno, S., 1995. Regulation of carbohydrate metabolism in fish:
585 metabolic response to acclimation temperature in carp. Fish. Sci. 61, 512–516.
586 Skiftesvik, A.B., Bjelland, R.M., Durif, C.M.F., Johansen, I.S., Browman, H.I., 2013.
587 Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus*
588 *bergylta*). Aquaculture 402–403, 113–118.
589 Torrecillas, S., Mompel, D., Caballero, M.J., Montero, D., Merrifield, D., Rodiles, A., Robaina,
590 L., Zamorano, M.J., Karalazos, V., Kaushik, S., Izquierdo, M.S., 2017. Effect of fishmeal
591 and fish oil replacement by vegetable meals and oils on gut health of European sea bass
592 (*Dicentrarchus labrax*). Aquaculture 468, 386–398.
593 US Department, NOAA of Commerce, Satellite Data and Information, National
594 EnvironmentalService, Office of Satellite and Product Operations, 1998. NOAA's Office
595 of Satellite and Product Operations [WWW Document]. URL
596 http://www.ospo.noaa.gov/Products/ocean/sst/monthly_mean.html (accessed 3.15.18).

Vågnes, Ø., Biering, E., Almås, K., 2014. Development of injection and cohabitation challenge models for atypical *Aeromonas salmonicida* in farmed ballan wrasse (*Labrus bergylta*), Veterinærinstituttets Rapportserie 1-2014. Norwegian Veterinary Institute; 2014.

Van Ham, E.H., Berntssen, M.H.G., Imsland, A.K., Parpoura, A.C., Wendelaar Bonga, S.E., Stefansson, S.O., 2003. The influence of temperature and ration on growth, feed conversion, body composition and nutrient retention of juvenile turbot (*Scophthalmus maximus*). Aquaculture 217, 547–558.

Yang, S.-D., Liou, C.-H., Liu, F.-G., 2002. Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). Aquaculture 213, 363–372.

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 ºC) 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.

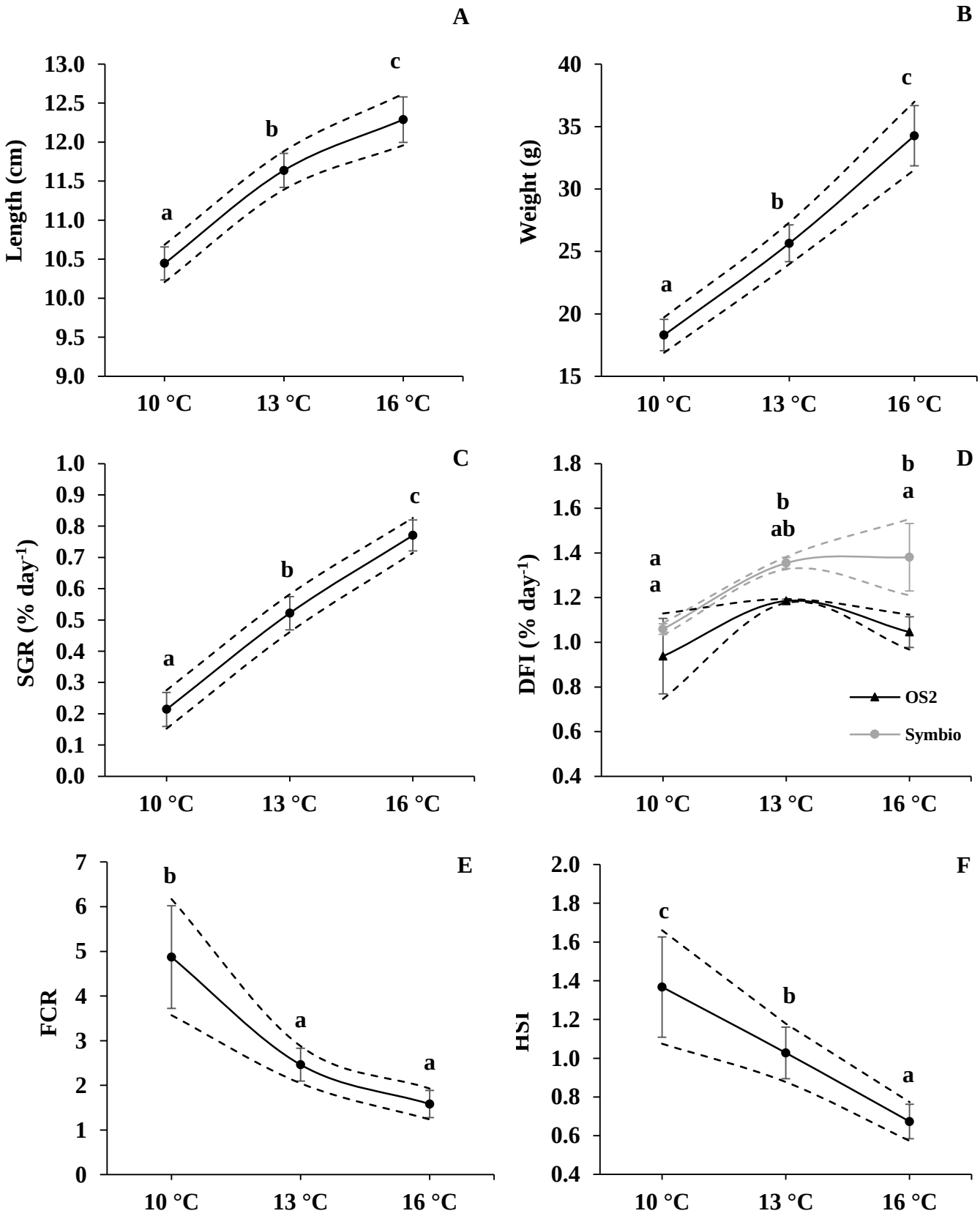

A
Length (cm)
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
a
b
c
10 °C
13 °C
16 °C
B
Weight (g)
40
35
30
25
20
15
a
b
c
10 °C
13 °C
16 °C
C
SGR (% day-1)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a
b
c
10 °C
13 °C
16 °C
D
DFI (% day-1)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
a
a
b
ab
b
a
OS2
Symbio
10 °C
13 °C
16 °C
E
FCR
7
6
5
4
3
2
1
0
b
a
a
10 °C
13 °C
16 °C
F
HSI
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
c
b
a
10 °C
13 °C
16 °C

Figure 2.

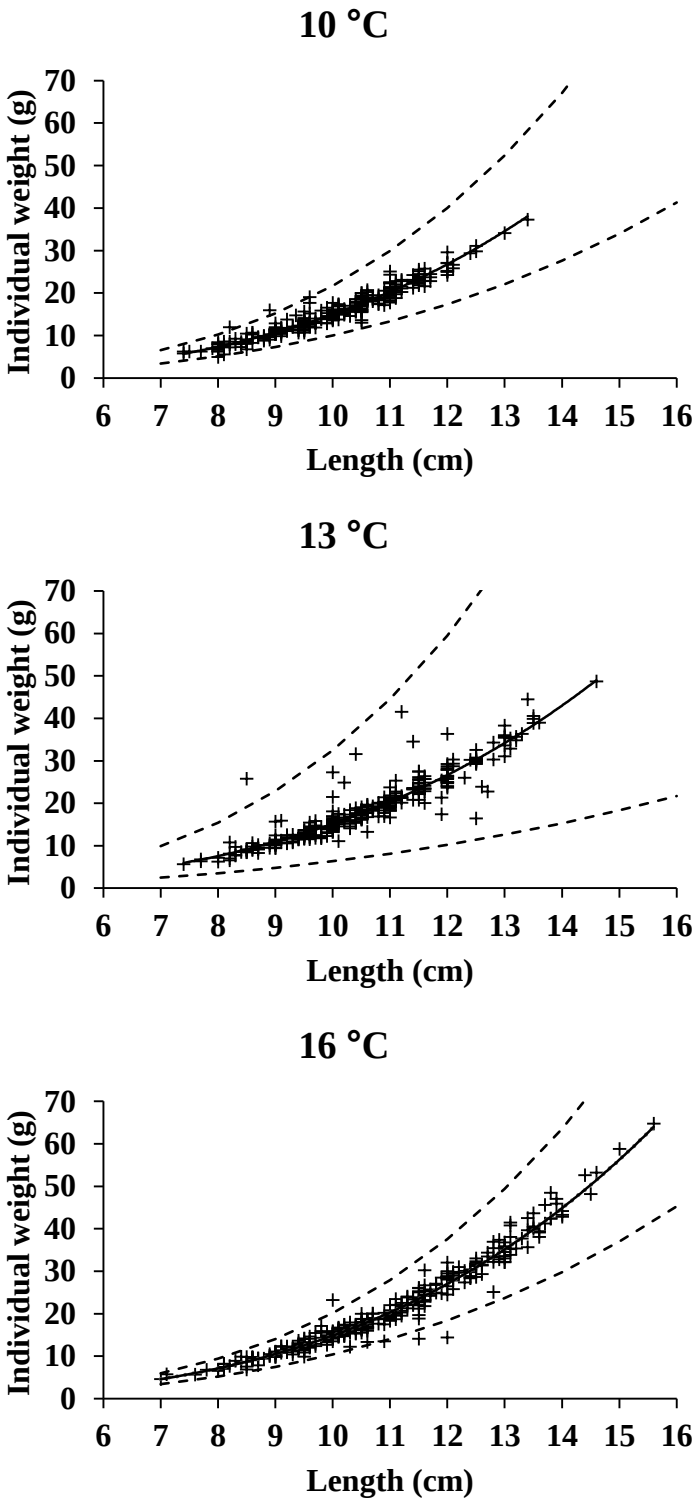
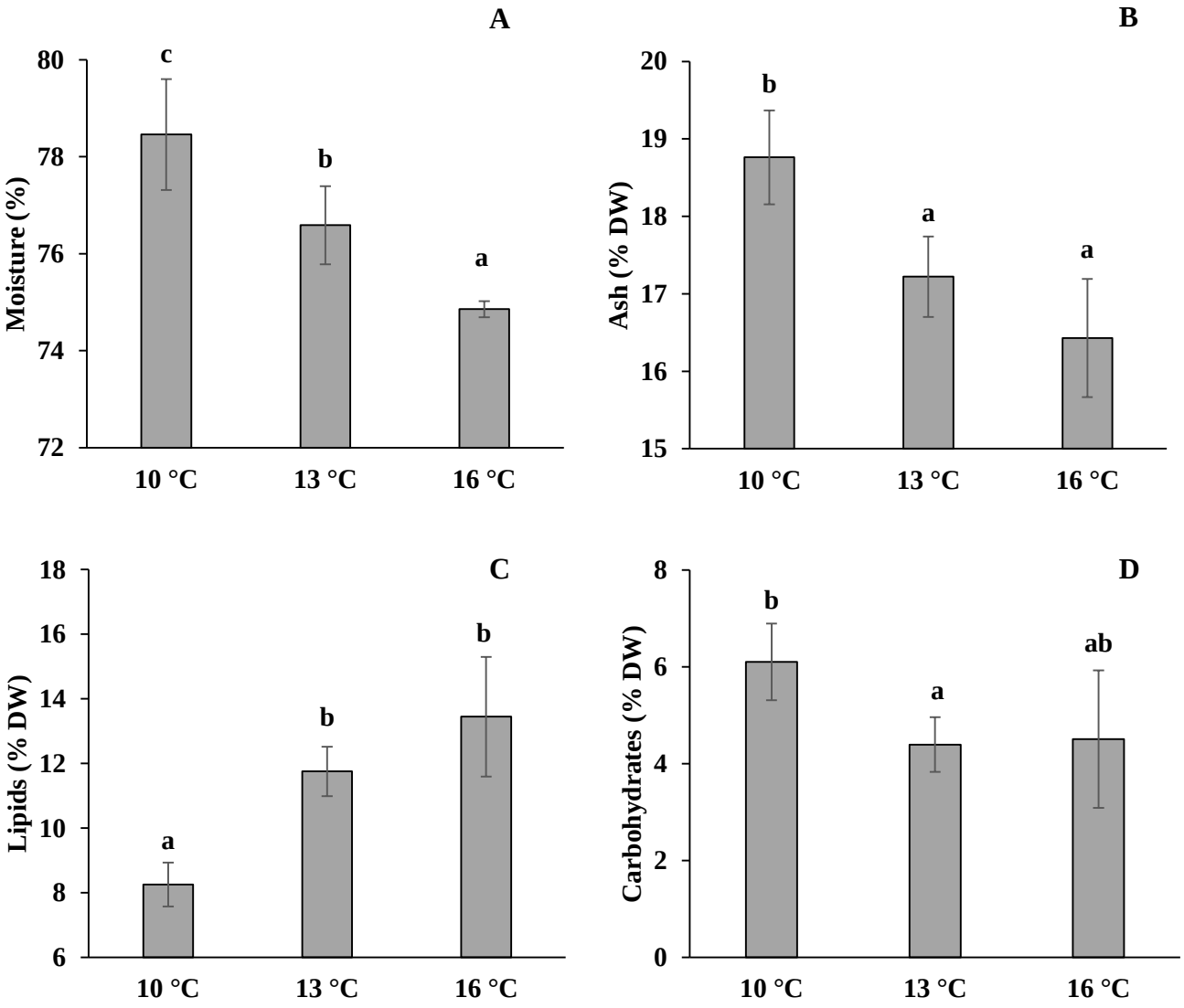

10 °C
Individual weight (g)
0
10
20
30
40
50
60
70
6
7
8
9
10
11
12
13
14
15
16
Length (cm)
13 °C
Individual weight (g)
0
10
20
30
40
50
60
70
6
7
8
9
10
11
12
13
14
15
16
Length (cm)
16 °C
Individual weight (g)
0
10
20
30
40
50
60
70
6
7
8
9
10
11
12
13
14
15
16
Length (cm)

Figure 3.


A
Moisture (%)
80
78
76
74
72
c
b
a
10 °C
13 °C
16 °C
B
Ash (% DW)
20
19
18
17
16
15
b
a
a
10 °C
13 °C
16 °C
C
Lipids (% DW)
18
16
14
12
10
8
6
a
b
b
10 °C
13 °C
16 °C
D
Carbohydrates (% DW)
8
6
4
2
0
b
a
ab
10 °C
13 °C
16 °C