Received: 3 April 2023 | Accepted: 14 June 2023

DOI: 10.1111/jfb.15482

REGULAR PAPER

JOURNAL OF FISH BIOLOGY fsbi

Welfare and performance of ballan wrasse (*Labrus bergylta*) reared at two different temperatures after a preparatory feeding trial with enhanced dietary eicosapentaenoic acid

Johanna S. Kottmann[1] | Gerd M. Berge[2] | Katerina Kousoulaki[3] | Tone-Kari Knutsdatter Østbye[4] | Elisabeth Ytteborg[5] | Bjarne Gjerde[6] | Ingrid Lein[1]

[1]Department of Aquaculture Production Technology, Nofima, Sunndalsøra, Norway

[2]Department of Nutrition and Feed Technology, Nofima, Sunndalsøra, Norway

[3]Department of Nutrition and Feed Technology, Nofima, Bergen, Norway

[4]Department of Nutrition and Feed Technology, Nofima, Ås, Norway

[5]Department of Fish Health, Nofima, Ås, Norway

[6]Department of Breeding and Genetics, Nofima, Ås, Norway

Correspondence
Johanna S. Kottmann, AquaGen AS, Havnegata 9, 7010, Trondheim, Norway.
Email: johanna.kottmann@aquagen.no

Funding information
Fiskeri-og havbruksnæringens forskningsfond, Grant/Award Number: 901563

Abstract

Concerns have long been raised about the welfare of ballan wrasse (*Labrus bergylta*) used for the biological control of sea lice in Atlantic salmon (*Salmo salar*) aquaculture. This study assessed the effect of increased dietary eicosapentaenoic acid (EPA) levels and initial condition factor (CF) on the subsequent performance and welfare of ballan wrasse farmed in high and low water temperatures. Fish were fed a diet with either commercial or high EPA levels for 3 months at 15°C. Subsequently, fish were tagged with a passive integrated transponder, measured for their CF and divided into two groups consisting of fish from both treatments and reared for 4.5 months at either 15 or 6°C fed a commercial diet. Each fish was categorized as high (≥2.7) or low CF (<2.7) fish based on the calculated average CF of the population. Dietary composition influenced the fatty acid (FA) profile of the stored lipids without affecting the growth or welfare of ballan wrasse. Fish reared at 15°C showed higher growth, more fat and energy reserves and less ash content. Fish reared at 6°C lost weight, using up their body lipids at the end of the temperature trial. Gene expression analyses showed upregulation of the positive growth marker (*GHrα*) and two genes involved in the synthesis and oxidation of FAs (*elovl5*, *cpt1*) and downregulation of the negative growth marker (*mstn*) in fish reared at 15°C compared to those reared at 6°C. Fish reared at 6°C showed upregulated levels of *il-6* compared to those reared at 15°C, suggesting an enhanced immune reaction in response to low temperature. Fish with high CF showed better survival, growth and performance compared to those with low CF. External welfare scoring showed higher prevalence and severity in emaciation, scale loss and the sum index score (of all measured welfare parameters) in fish reared at 6°C compared to those reared at 15°C and better welfare in fish with high CF compared to those with low CF. Histological examination of the skin showed that fish reared at 6°C had decreased epidermal thickness, a lower overall number of mucous cells in the inner and outer epidermis and a different organization of mucous cells compared to fish reared at 15°C, indicating stress in fish reared at 6°C. Overall, low water temperatures had profound effects on the performance and external and

internal welfare parameters of ballan wrasse and can be considered a stressor likely affecting the delousing efficacy. These findings support the seasonal use of different cleaner fish species. High CF, but not increased dietary EPA levels, appeared to help fish cope better with low water temperatures and should thus be assessed and considered before deploying them in salmon cages.

KEYWORDS
cleaner fish, dietary fatty acids, gene expression, *Labrus bergylta*, rearing temperature, skin health

1 | INTRODUCTION

Finding sustainable solutions to overcome the issue of the ectoparasitic salmon louse (*Lepeophtheirus salmonis*) is one of the major challenges for the salmon (*Salmo salar*) farming industry (Barrett *et al*., 2020; Costello, 2009; Torrissen *et al*., 2013). The deployment of cleaner fish has developed as one of the most widely used approaches to control sea lice (Brooker *et al*., 2018; Overton *et al*., 2020). In 2020, about 50 million cleaner fish were deployed at Norwegian salmon farms, the majority consisting of two main species, ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) (Norwegian Directorate of Fisheries, 2021). Nonetheless, there are increasing ethical concerns about challenges in production and welfare issues with cleaner fish (Brooker *et al*., 2018; Mo & Poppe, 2018; Powell *et al*., 2018). Among known welfare challenges for cleaner fish are high mortality (Overton *et al*., 2020), prevalence of deformities (Cavrois-Rogacki *et al*., 2021; Fjelldal *et al*., 2020) and susceptibility to diseases (Brooker *et al*., 2018; Powell *et al*., 2018). If continued, future use and research must have increased focus on optimizing hatchery production to produce robust cleaner fish adapted for environmental conditions in salmon cages to ensure high welfare and delousing efficacy.

The two main cleaner fish species vary profoundly in terms of biology and life history and show differences with regard to thermal tolerance. Lumpfish is a semi-pelagic cold water species that can tolerate low temperatures and is thus often the preferred cleaner fish to be stocked during winter months (Geitung *et al*., 2020; Hvas *et al*., 2018). In contrast, ballan wrasse are known to remain in deeper and warmer waters while entering a dormant state with low activity and metabolic rates in colder periods (Morel *et al*., 2013; Noble *et al*., 2019; Yuen *et al*., 2019). Anecdotal reports from Norwegian fish farmers state that ballan wrasse become dormant around 6°C. This might lead to their low delousing efficacy during low temperatures (Yuen *et al*., 2019) and susceptibility to predation. It has been suggested to best deploy ballan wrasse in spring months with increasing water temperatures (Brooker *et al*., 2018). This is in agreement with results from Cavrois-Rogacki *et al*. (2019), who demonstrated increasing growth in ballan wrasse with increasing temperatures (10, 13 and 16°C). The authors suggest that the optimal temperature for growth in this species might be even higher.

Temperature greatly affects the physiology and biochemistry of ectothermic organisms such as fish in terms of metabolic response (Guderley, 2004), growth factors (Gabillard *et al*., 2005), immune response (Bowden *et al*., 2007) or gene expression profiling (Malek *et al*., 2004). Fatty acid (FA) metabolism is one of the key mechanisms to cope with thermal stress (Johnston & Dunn, 1987; Tocher *et al*., 2004), and changes in FA composition under cold stress have been shown for common carp (*Cyprinus carpio* L.) (Trueman *et al*., 2000), rainbow trout (*Oncorhynchus mykiss*) (Hazel, 1979), milkfish (*Chanos chanos*) and grass carp (*Ctenopharyngodon idella*) (Hsieh & Kuo, 2005).

Diets for ballan wrasse have been formulated based on previous studies on the nutritional requirements and special digestive physiology and raw material preferences of the species (Hamre *et al*., 2013; Kabeya *et al*., 2018; Kousoulaki *et al*., 2015, 2021). Kabeya *et al*. (2018) characterized the metabolic pathways, concluding that dietary arachidonic acid (ARA, 20:4 n-6) and eicosapentaenoic acid (EPA, 20:5 n-3) are essential to meeting the FA requirements of this species. Although it is expected that ballan wrasse can biosynthesize docosahexaenoic acid (DHA) from EPA to some extent, it is suggested that dietary DHA (22:6 n-3) is required as well (Kabeya *et al*., 2018). For ballan wrasse larvae, a higher dietary EPA:DHA ratio has been found to be associated with higher growth performance (Kousoulaki *et al*., 2015).

Unpublished results show a correlation between condition factor (CF) and the amount of intestinal fat reserves. Therefore, the aim of the study was to evaluate whether enhanced dietary EPA during a preparation period and differences in CF affect mortality, performance and welfare of ballan wrasse kept at two different temperatures. First, fish were fed two different diets varying in EPA content and reared at 15°C. In the second part of the experiment, fish fed both diets and with high or low initial CF were reared at 6 or 15°C. The temperature treatments were chosen based on experience from the industry, where 15°C is the standard regime for Norwegian ballan wrasse production, whereas 6°C is the temperature commonly found in salmon sea cages in Norway during the winter months. In the present study, the effect of dietary EPA, CF, and temperature on survival, growth, chemical and FA composition, welfare scoring, skin histology and gene expression on growth, metabolism and stress-related genes was assessed.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The experiment was approved by the Norwegian Food Authority (FOTS ID 25965) under the Norwegian regulation for use of animals

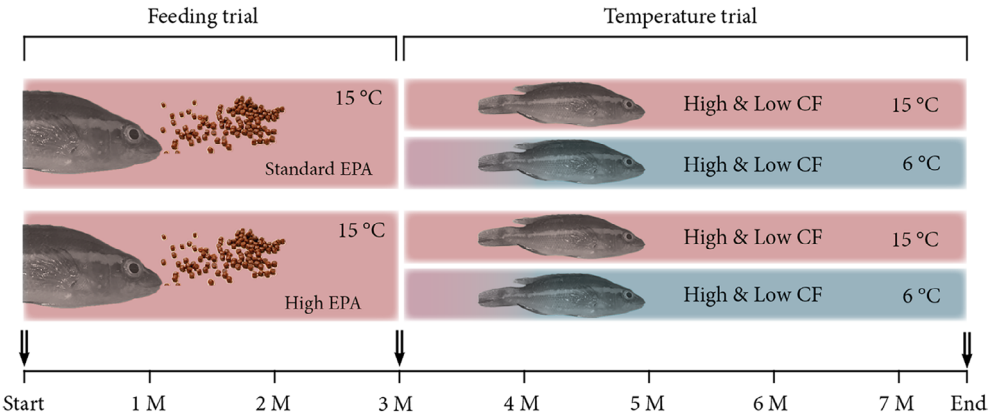

FIGURE 1 Experimental design consisting of feeding and temperature trials. The timeline indicates the start and end as well as the experimental duration in months (M). Arrows indicate sampling points. The number of fish at the start of the feeding trial was 280 fish per tank, and that at the start of the temperature trial was 140 fish per tank

for experimental purposes, "FOR-2015-06-18-761 Forskrift om bruk av dyr i forsøk." This is a national adaptation of (and in compliance with) the "Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes."

2.2 | Dietary treatments and feed formulation

The experimental diets were produced at the Aquafeed Technology Center of Nofima in Bergen, Norway. The formulation of the two diets is given in Supporting Information Table S1. The EPA levels in the standard-EPA diet were kept similar to commercially available diets (1.2%–1.6% of the diet, own analyses), whereas the EPA levels in the high-EPA diet were increased by replacing fish oil with an EPA concentrate (EPAX 6015 TG/N, EPAX Norway AS, Ålesund, Norway) in the diet formulation.

2.3 | Experimental design

The experiment consisted of two parts: (a) a feeding trial preparing fish with different chemical compositions and CFs for (b) a subsequent temperature trial. The experimental design is shown in Figure 1. Experimental fish were produced by Havlandet Marin Yngel, Florø, Norway and reared at a SalMar ongrowing site at Tjeldbergodden, Norway. Fish was transported to the Nofima Aquaculture Research Center in Sunndalsøra, Norway, in December 2020. After transport, fish were acclimatized to the new environment for 6 weeks and were fed a commercial diet (OTOHIME S2, PTAqua, Dublin, Ireland) during the acclimatization period. At the start of the experiment, the mean fish weight was 37.0 ± 0.6 g. Fish were randomly distributed into 6 × 400 l flat bottom tanks with black walls in a flow-through system with sea water filtered to 20 µm and UV treated with a flow of 15 l per minute per tank. Oxygen (O_2) was added to holding tanks before the water entered the experimental fish tanks, and the O_2 level was recorded continuously. In addition, the O_2 levels in fish tank outlets were measured using a hand-held meter (Oxygard Handy Polaris C, Sterner Aqua AS) twice a week to ensure that the oxygen level in the fish tanks did not go below 80%. Temperature and salinity were measured daily. Each fish tank was equipped with an LED light dimmed to 9%, and fish were kept at 24 h light regime, as commonly used in the Norwegian industry. Throughout the whole experimental period, all tanks were equipped with shelters (artificial kelp) to provide hiding places for the fish. This has shown to increase survival during winter times in sea cages (Treasurer, 2002). Each tank was stocked with 280 fish, and tanks were randomly assigned treatments.

During the feeding trial, fish in three tanks were fed an experimental diet with EPA levels similar to commercially available diets for ballan wrasse (standard EPA), whereas fish in the remaining three tanks received an experimental diet with enhanced polyunsaturated fatty acid (PUFA) levels (high EPA). The feed was distributed using belt feeders with 96 feedings per day. The feeding rations were adjusted according to apparent appetite, based on the feed leftovers observed at the bottom of the tank, aiming at a low but constant level of leftover feed to ensure optimal voluntary feed intake. Due to the required consistency of the pellets for ballan wrasse, it is not possible to use standard feed waste collection methods. The mean temperature in the tanks during the feeding trial was 15.1 ± 0.2°C, salinity 32.5 ± 0.3 PSU and mean O_2 saturation 91.04% ± 3.28%. Shortly before the end of the feeding trial, all tank populations were reduced to 200 fish per tank as fish were growing faster than estimated based on previous experience and lower mortality occurred than estimated beforehand. This measure to reduce tank biomass was taken to ensure high oxygen values throughout the whole experimental period. The initial feeding trial lasted for 3 months and was followed by a subsequent 4.5-month temperature trial. At the end of the feeding trial, fish were tagged with a passive integrated transponder (PIT tag), and length and weight were measured to calculate the CF of individual fish using the formula: body weight, g/(standard length, cm)^3 × 100. Fish with a CF ≤ 2.69 were considered to have a low CF, whereas CF ≥ 2.70 was considered high. The threshold was defined based on a previously collected sub-sample of 15 fish randomly sampled from each of the six tanks used in the feeding trial (a total of 90 fish, 45 per diet). These recordings were used to calculate the average CF.

After the termination of the feeding trial, 140 individually tagged fish from both dietary treatments were distributed to each of the six new tanks. Each tank received similar amounts of fish from each diet and with high and low CFs. During the temperature trial, the

temperature of the three tanks was continued to maintain at 15°C, whereas that of the other three tanks was gradually decreased to 6°C, lowering 2°C per week. The temperature of the incoming water was adjusted by adding colder water to a holding tank. Again, temperature and salinity were measured daily and O_2 twice per week. Mean measured temperatures during the temperature trial were 15.1 ± 0.3°C for the high-temperature treatment and 5.8 ± 0.3°C for the low-temperature treatment (after temperature reduction was completed). The mean salinity was 32.6 ± 0.5 PSU, and the mean O_2 saturation was 95.7% ± 4.5%. During the temperature trial, all fish received the commercial OTOHIME S2 (PTAqua) diet.

2.4 | Data collection

Ballan wrasses were sampled at the start of the experiment, at the transition between the feeding and the temperature trial (after 3 months, mid-sampling) and at the end of the experiment (*c.* 7.5 months, final sampling). Mortality was recorded daily throughout the whole experimental period, and survival rates were calculated subsequently. Before sampling, fish were euthanized with Finquel (250 mg l^{-1}).

2.4.1 | Start of sampling

Ninety fish were sampled randomly from the holding tanks and frozen at −20°C for whole-body (WB) chemical composition analyses (lipid, protein, energy, dry matter and ash content) before distributing them to the experimental tanks. Finally, the fish in each tank were bulk-weighed. The mean start weights were used for the calculation of the specific growth rate (SGR) during the feeding trial.

2.4.2 | End of feeding trial

At the end of the feeding trial, weight and length were measured for all fish (950 fish in total). Moreover, 120 fish (20 fish per tank, 10 per high and low CFs) were sampled and dissected, and the following additional parameters were measured: liver weight, intestinal weight, intestinal fat weight and gutted weight. In addition, 180 fish (30 random fish per tank) were sampled for external welfare scoring and subsequently frozen at −20°C. A pooled sample of five fish with high CF and five fish with low CF per tank was analysed for chemical composition (lipid, protein, energy, dry matter, ash and FA analyses).

2.4.3 | End of temperature trial

At the end of the temperature trial, the final sampling, all remaining fish were measured again for length and weight (746 fish). Moreover, 240 fish (40 fish per tank, 10 per diet and high and low CFs) were sampled and dissected for the same parameters as described earlier for the mid-sampling. In addition, skin samples for histological analyses were preserved in formalin. The samples were kept at room temperature for 24 h and subsequently stored at 4°C. Also, a piece of liver was sampled in RNAlater for gene expression analyses. The samples were stored at 4°C for 24 h and subsequently frozen at −20°C. Samples for skin histology and gene expression were taken only for 96 fish in total (16 fish per tank, 4 per diet and high and low CFs). Finally, 240 fish (40 fish per tank, 10 per diet and high and low CFs) were sampled for external welfare scoring and subsequently frozen at −20°C. Two of these samples had to be taken out, leading to a total of 238 fish used for welfare scoring. A pooled sample of five fish with high and five fish with low CF per diet and tank was analysed for chemical composition (lipid, protein, energy, dry matter, ash and FA analyses).

Based on the fish weight data, the following growth rates were calculated:

$$\text{Thermal growth coefficient (TGC)} = \left(W_2^{1/3} - W_1^{1/3}\right) \times (1000/d^{\circ})$$

$$\text{Specific growth rate (SGR)}, \%/\text{day} = (\ln W_2 - \ln W_1) \times (t)^{-1} \times 100$$

W_1 and W_2 are average body weight (g) at the start and end of the trial, respectively, d° is the sum of day degrees during the trial and t is the number of days in the trial.

In addition, the following parameters were calculated:

$$\text{Hepatosomatic index (HSI)} = (\text{liver weight}/\text{whole fish weight}) \times 100$$

$$\text{Intestinal fat index (IFI)} = (\text{intestinal fat weight}/\text{whole fish weight}) \times 100$$

2.5 | Chemical composition

The experimental diets were analysed for dry matter (105°C, until stable weight), ash (550°C, until stable weight), lipid (Bligh & Dyer, 1959), FA profile (AOCS Ce 1b-89) and nitrogen (AOAC 2001.11; Kjeltec 8400 Analyser Unit, Tecator, Höganäs, Sweden), and nitrogen-free extract (NFE) was calculated as a difference (NFE = dry matter − ash − lipid − crude protein). Whole fish (five fish pooled per tank and treatment) were analysed for dry matter, ash, lipid, FA profile and nitrogen following the same methods used for feed samples. Nitrogen was further calculated to crude protein (N × 6.25). In addition, whole fish samples were analysed for gross energy (Parr 63000 Bomb calorimeter, Moline, IL, USA).

2.6 | Welfare traits

A total of 12 welfare traits were subjectively scored following the RENSVEL scheme for ballan wrasse (Espmark *et al.*, 2019) using a score from 0 to 3 with 0, no damage; 1, minor damage; 2, moderate damage; and 3, major damage. The traits were as follows: operculum damage, snout damage, upper jaw deformities, lower jaw deformities,

emaciation, vertebral deformities, scale loss and fin damage on the dorsal fin, caudal fin, pectoral fin, anal fin and pelvic fin. In addition, for each individual, an overall index trait was calculated as the sum of the score of all 12 traits.

2.7 | Gene expression

Total RNA was extracted using a Biomek 4000 Automated Workstation (Beckman Coulter, Indianapolis, IN, USA) and applying the Agencourt® RNAdvance tissue kit (Agencourt Bioscience Corporation, Beverly, MA, USA) according to the manufacturer's instructions. Potential trace levels of DNA were eliminated by the treatment of RNA with DNase I (Invitrogen, Carlsbad, CA, USA). RNA concentration and purity were analysed by spectrophotometry using Nanodrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) and a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

cDNA was synthesized from the resulting total RNA using TaqMan® Reverse Transcription Reagents kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. The quantitative PCR (qPCR) reaction was performed by mixing 4 µl of diluted (1:10) cDNA, 1 µl of forward and reverse primers (final concentration of 0.5 µM) and 5 µl of PowerUp SYBR Green Master Mix (Applied Biosystems). The efficiencies of the primers were tested by including a standard curve for each primer pair. All samples were analysed in parallel, and non-template and non-enzyme controls were included. The qPCR was performed using QuantStudio 5 instrument (Thermo Fisher Scientific, Waltham, MA, USA) under the following conditions: 95°C for 20 s, 40 cycles at 95°C for 1 s and 60°C for 20 s, 95°C for 1 s and 60°C for 20 s and 95°C for 1 s.

Primers of six genes were retrieved from previous studies, whereas those of the remaining five genes were designed using Primer-BLAST (National Center for Biotechnology Information) (Supporting Information Table S2). The specificity of the designed primers was confirmed by sequencing (Sanger sequencing, supreme run, Eurofins Genomics, Huntsville, AL, USA). β-Actin and ef1a were evaluated as reference genes using RefFinder (Xie *et al.*, 2012). The relative gene expression level was calculated following the ΔΔCt method with efficiency correction (Pfaffl, 2004) using β-actin as the reference gene.

2.8 | Histology

Skin samples fixed in buffered 4% formalin were carefully dissected, orientated and placed in tissue-embedding cassettes (Simport, Beloeil, Quebec, Canada). The samples were dehydrated through 100% alcohol and then in a clearant Xylene bath, using an automated tissue processor (TP1020, Leica Biosystems, Nussloch GmbH, Nussloch, Germany), before being infiltrated in melted 60°C paraffin (Merck KGaA, Darmstadt, Germany). Paraffin-embedded tissue samples were cut into 5 µm sections using a microtome (Leica RM 2165, Leica Biosystems, Nussloch GmbH, Nussloch, Germany), mounted on polysin-coated slides (VWR, Avantor, Radnor, PA, USA) and dried overnight at 37°C. The sections were deparaffinized and rehydrated, and staining was performed using an automated special stainer (Autostainer XL Leica Biosystems, Nussloch GmbH). Paraffin sections were stained with Alcian Blue Periodic Acid Schiff (pH 2.5, Alcian Blue 8GX, Sigma Aldrich, Darmstadt, Germany). Stained samples were scanned using a light microscope Leica slide scanner and evaluated in Aperio Image Scope (Leica Microsystems, Wetzlar, Germany).

Measurements for the skin were made in a region of *c.* 1000 µm per section, at two different positions in each sample: outer and inner epidermis. Scales are overlapping in fish skin, making the outer epidermis on top of the scale in close contact with the environment and the inner epidermis covered by the scale. In each region, epidermal mucous cells were counted and defined as either "outer" (those touching the outer border of the epidermis) or "total" (the total number of mucous cells in the epidermis). Epidermal thickness was measured in five different locations of the selected region. Some samples were lacking the outer epidermis, making them unsuitable for histological analyses, and were therefore removed. Removing these samples left $n = 27$ in the high CF group and $n = 14$ in the low CF group.

2.9 | Statistical analyses

Data were analysed using SAS/STAT Software (2002–2012). An overview of the obtained data sets and statistical models used to analyse the data is given in Supporting Information Table S3.

2.9.1 | Feeding trial

The magnitude of the effect of diet on the studied traits in data sets 1 and 2 was determined from the following linear mixed model using the PROC MIXED procedure in SAS (Model 1).

$$y_{ijk} = M + D_i + t_{ij} + e_{ijk}$$

where y_{ijk} is the recorded animal trait, M is the overall mean, D_i is the fixed effect of diet with two levels ($i = 1, 2$), t_{ij} is the random effect of the tank with three levels within diet ($j = 1, 2, 3$), and e_{ijk} is the random residual error of fish k within tank ij.

The magnitude of the effect of diet on the studied traits in data sets 3 and 4 was determined using a similar model without the tank effect (Model 2) as for these traits there was only one trait value per tank of the pooled WB sample of five fish per tank, and the random residual error (e_{ij}) is that for tank j within diet i.

The effect of diet on the trait SGR during the feeding trial was further tested using a *t*-test. The traits body weight and IFI were additionally analysed using a two-way ANOVA with the fixed effects of diet and CF and their interaction.

2.9.2 | Temperature trial

The magnitudes of the fixed effect of temperature and the carry-over effect of the fixed effects of diet and CF from the feeding trial on the

studied traits in data sets 5, 6, 7 and 8 were determined from the following linear mixed model using the PROC MIXED procedure in SAS (Model 3):

$$y_{ijklm} = M + T_i + D_j + C_k + TD_{ij} + TC_{ik} + DC_{jk} + t_{il} + e_{ijklm}$$

where y_{ijklm} is the recorded animal trait; T_i is the fixed effect of temperature with two levels ($i = 1, 2$); D_j is the fixed effect of diet j in the feeding trial with two levels ($j = 1, 2$); C_k is the fixed effect of CF at the end of the feeding trial with two levels (high and low); TD_{ij}, TC_{ik} and DC_{jk} are the fixed interaction effects between pairs of the main effects; t_{il} is the random effect of tank l with three levels ($l = 1, 2, 3$) within temperature ($j = 1, 2, 3$); and e_{ijklm} is the random error of fish m within tank il ($m = 1, 2, \ldots$ number of recorded fish within the tank).

The magnitudes of the effect of diet and the carry-over effect of the fixed effects of diet and CF from the feeding trial on the studied traits in data sets 9 and 10 were determined using a similar model without the tank effect (Model 4) as for these traits there was only one trait value per tank of the pooled WB sample of 2×5 fish per tank, and the random residual error (e_{ij}) is that for tank j within diet i.

For each of the traits in the 11 different data sets, estimates of the difference between the two diets (feeding trial) and the difference between the two temperatures, the two diets and the two levels of the CF (temperature trial) were obtained from single degree-of-freedom contrast provided by the PROC MIXED procedure. The relative importance of each effect in the different models was expressed as the sum of squares of the effect, as a percentage of the total sum of squares obtained from the PROC GLM procedure in SAS, because the PROC MIXED procedure does not provide the sum of squares as it is based on a maximum likelihood approach.

Due to the low number of samples ($n = 41$), the statistical analyses of histological data included only the fixed effects of temperature with two levels (6 and 15°C) and the fixed effect of CF with two levels (high and low), thus omitting the effect of diet. These data were also analysed with the PROC MIXED procedure in SAS with the error term as the only random effect in Model 5.

3 | RESULTS

3.1 | Start of sampling

At the beginning of the feeding trial, the chemical composition of whole fish analyses showed a dry matter of 26.77% ± 2.23%, ash content of 5.43% ± 0.8%, fat content of 4.3% ± 2.29%, crude protein of 17.1% ± 0.7% and energy content of 5.56 ± 1.10 MJ kg^{-1}.

3.2 | Feeding trial

3.2.1 | Experimental diets

The chemical composition and FA profiles of the two experimental diets from the feeding trial, as well as the commercial diet OTOHIME S2 used in the temperature trial, are given in Supporting Information Table S4. Because fish oil was replaced by the omega-3 concentrate EPAX 6015 TG/N in high-EPA diet, this diet had higher levels of PUFA, in particular, n-3 PUFA levels, compared to standard-EPA diet, which had the highest levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). FA levels in OTOHIME S2 were closer to standard EPA than to high EPA. Nonetheless, higher SFA and n-3 PUFA levels were found in OTOHIME S2 compared to standard EPA, whereas standard EPA showed higher levels of MUFA and n-6 PUFA.

3.2.2 | Performance and chemical composition

Results on growth and performance during the feeding trial are presented in Table 1. Overall, survival during the feeding trial was high, and no differences ($P > 0.05$) were found for fish fed standard-EPA and high-EPA diets. The dietary treatment further did not impact weight and length or CF of the fish ($P > 0.05$). Moreover, there was no influence of dietary regime on the HSI and IFI measured at the end of the feeding trial ($P > 0.05$). Furthermore, there was no significant difference between SGR for fish fed standard-EPA (0.49 ± 0.01) and high EPA (0.50 ± 0.04; $P > 0.05$) diets.

3.2.3 | FA composition

The FA composition of whole fish at the end of the feeding trial after being fed standard-EPA and high-EPA diets is presented in Table 3. The FA levels in fish at this stage reflected the FA composition of the diets, whereas fish in the high EPA treatment had in their body as compared to the diet they were fed increased SFA (22% in WB FA *vs.* 14% in the diet) and MUFA (32% in WB FA *vs.* 23% in the diet) and decreased n-3 PUFA levels (34% in the WB FA *vs.* 48% in the diet), mostly due to a reduction of WB EPA (18% in WB FA *vs.* 30% in the diet).

3.2.4 | Welfare scoring

The welfare scoring at the end of the feeding trial showed no significant effect of the dietary regime on emaciation and the sum index of all 12 measured parameters ($P > 0.05$). Nonetheless, there was a significant effect of diet ($P = 0.042$) on scale loss, with a higher prevalence of scale loss in fish fed a standard-EPA compared to high-EPA diet.

3.3 | Temperature trial

3.3.1 | Performance and chemical composition

Data on the performance and chemical composition of fish during the temperature trial are presented in Table 4, and the statistical results

TABLE 1 Survival during the feeding trial period and means and standard deviations (S.D.) of the traits recorded at the end of the feeding trial for ballan wrasse (*Labrus bergylta*) fed standard-EPA or high-EPA diets and difference between the least squares means of the two diets with significance *P*-values from the applied statistical model 1

	Standard EPA		High EPA			
Trait	**Mean**	**S.D.**	**Mean**	**S.D.**	**Difference of least squares means**	***P*-value diet**
Survival (%)	93.9	2.8	95.7	2.0	−0.20 ± 0.10	>0.05
Final body weight (g)	60.7	21.2	61.3	20.7	−0.50 ± 1.36	>0.05
Standard length (cm)	12.9	1.3	12.9	1.4	−0.04 ± 0.09	>0.05
CF	2.73	0.28	2.74	0.28	−0.002 ± 0.02	>0.05
HSI (%)	1.33	0.53	1.23	0.41	0.10 ± 0.09	>0.05
IFI (%)	0.95	0.69	0.91	0.63	0.03 ± 0.12	>0.05

Abbreviations: CF, condition factor; EPA, eicosapentaenoic acid; HSI, hepato-somatic index; IFI, intestinal fat index.
Note: Finally, the chemical composition measured in fish at the end of the feeding trial did not differ significantly among dietary regimes (Table 2; $P > 0.05$).

of these parameters are given in Table 5. Overall, survival during the temperature trial was high. There was lower survival in one of the tanks, leading to a significant tank effect ($P = 0.008$) in the statistical model, which also showed a low level of explanation (R^2). Nonetheless, a significant effect of CF on survival was found with higher survival of fish with high CF compared to those with low CF. Temperature strongly affected SGR and TGC with positive growth rates at 15°C and negative growth rates at 6°C. Furthermore, TGC was, to a lower extent, affected by CF and the interaction of temperature and CF, with higher growth in fish with high CF, in particular, at 15°C. Altogether, this led to significantly higher body weight and length in fish reared at 15°C and with high CF. There was a significant effect of diet on HSI; nonetheless, the overall model showed a very low explanation level (R^2). Temperature, CF and temperature × diet had a significant effect on the amount of intestinal fat in the fish. Nonetheless, temperature explained by far most of the observed differences, with fish reared at 15°C having more fat around the intestine than fish reared at 6°C. Except for survival, no tank effect was found for any of the measured parameters ($P > 0.05$).

To analyse the effect of CF on fish entering the temperature trial, weight and IFI were additionally compared between high and low CFs. Fish with high CF (69.1 ± 2.8 g) had a higher weight than fish with low CF (51.4 ± 3.1 g) independent of the dietary regime ($P < 0.0001$). The IFI for ballan wrasse at the end of the feeding trial and at the end of the temperature trial is shown in Figure 2. At the end of the feeding trial, the IFI was twice as high in fish with high CF (1.2 ± 0.6%) compared to that in fish with low CF (0.61 ± 0.5%) ($P < 0.0001$; Figure 2a). No significant effect of diet or diet × CF interaction was found ($P > 0.05$). This trend is confirmed at the end of the temperature trial, where temperature additionally had a strong effect on the intestinal fat content, as described earlier (Figure 2b).

The chemical composition of whole fish sampled at the end of the temperature trial is presented in Table 6, and the statistical results are given in Table 7. At the end of the temperature trial, dietary regime during the feeding trial did not affect dry matter, ash, fat, crude protein or energy ($P > 0.05$). Nonetheless, temperature and CF significantly affected the dry matter, ash, fat, crude protein and energy content of the fish. Here, the temperature regime explained the largest proportion of differences, except for ash, where temperature and CF explained the difference to the same extent. Fish reared at 15°C showed higher levels of dry matter, fat, crude protein and energy compared to those reared at 6°C. In contrast, ash content was higher in fish reared at 6°C compared to those reared at 15°C. No tank effect was found for any of the measured parameters of chemical composition ($P > 0.05$).

3.3.2 | FA composition

The FA composition of whole fish sampled at the end of the temperature trial is presented in Table 8, and the statistical analyses for each FA are given in Table 9. The dietary regime from the feeding trial had a significant effect on all FAs except for 18:2 n-6 and DHA. Moreover, the temperature had a significant effect on the SFAs, in particular, 14:0. Here, fish reared at 15°C showed higher levels compared to those reared at 6°C. Moreover, temperature treatment showed an effect on n-6 PUFA, in particular, 18:2 n-6, and on DHA, with fish reared at 15°C having lower levels compared to those reared at 6°C. CF had only a low influence on the FA composition at the end of the temperature trial. No tank effect was found for any of the FA analyses ($P > 0.05$).

3.3.3 | Welfare scoring

For each of the 13 welfare traits, the R^2 of Model 3 in Supporting Information Table S3 at the end of the temperature trial was as follows: operculum damage, 0.081; snout damage, 0.075; upper jaw deformities, 0.055; lower jaw deformities, 0.097; emaciation, 0.111; vertebral deformities, 0.069; scale loss, 0.220; dorsal fin damage, 0.032; caudal fin damage, 0.057; pectoral fin damage, 0.006; anal fin damage, 0.075; pelvic fin damage, 0.025; and sum index, 0.093. Due to the relatively low R^2 value for most of these welfare traits, more detailed results were reported only for the traits scale loss, emaciation

TABLE 2 Means and S.D. of the chemical composition of whole fish samples of ballan wrasse (*Labrus bergylta*) fed a standard-EPA or a high-EPA diet and differences between the least squares means of the two diets with significance *P*-values from the applied statistical model 2

	Start		Standard EPA		High EPA			
Trait	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Difference of least squares means**	***P*-value diet**
Dry matter (%)	26.77	2.23	28.45	0.80	27.79	1.83	0.67 ± 0.82	>0.05
Ash (%)	5.43	0.8	4.39	0.62	4.80	1.24	−0.42 ± 0.56	>0.05
Fat (%)	4.3	2.29	6.69	1.08	5.90	2.16	0.79 ± 0.99	>0.05
Crude protein (N × 6.25) (%)	17.1	0.7	17.32	0.36	17.18	0.69	0.02 ± 0.05	>0.05
Energy (MJ kg^{-1})	5.56	1.10	6.60	0.44	6.26	1.02	0.34 ± 0.45	>0.05

TABLE 3 Means and S.D. of the fatty acid composition (% of total fatty acids) of whole fish samples of ballan wrasse (*Labrus bergylta*) fed a standard-EPA or a high-EPA diet and differences between the least squares means of the two diets with significance *P*-values from the applied statistical model 2

	Standard EPA		High EPA			
Fatty acid	**Mean**	**S.D.**	**Mean**	**S.D.**	**Difference of least squares means (±S.E.)**	***P*-value diet**
14:0	5.20	0.1	4.0	0.3	1.27 ± 0.15	**<0.0001**
16:0	15.8	0.4	13.6	0.8	2.17 ± 0.37	**0.0002**
18:0	3.60	0.2	3.9	0.2	−0.30 ± 0.10	**0.012**
SFA	24.7	0.5	21.6	0.9	3.08 ± 0.43	**<0.0001**
16:1 n-7	6.0	0.1	4.8	0.3	1.18 ± 0.12	**<0.0001**
18:1	23.5	0.5	21.9	0.6	1.62 ± 0.30	**0.0003**
20:1	5.7	0.2	3.3	0.2	2.33 ± 0.13	**<0.0001**
22:1	4.4	0.3	1.5	0.1	2.92 ± 0.11	**<0.0001**
MUFA[a]	40.1	0.7	32.0	1.0	8.07 ± 0.50	**<0.0001**
18:2 n-6	5.0	0.2	5.0	0.1	0.02 ± 0.07	>0.05
20:4 n-6 (ARA)	0.8	0.1	1.7	0.2	−0.85 ± 0.08	**<0.0001**
n-6 PUFA[b]	6.6	0.2	7.5	0.1	−0.97 ± 0.09	**<0.0001**
20:5 n-3 (EPA)	8.6	0.2	17.7	1.1	−9.10 ± 0.46	**<0.0001**
22:5 n-3	1.4	0.1	2.1	0.2	−0.75 ± 0.08	**<0.0001**
22:6 n-3 (DHA)	10.5	0.4	10.9	0.7	−0.43 ± 0.32	>0.05
n-3 PUFA[c]	23.5	0.6	34.2	1.6	−10.73 ± 0.68	**<0.0001**

Note: Significant *p*-values have been highlighted in bold.
Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
[a]Includes C24:1 n-9.
[b]Includes C20:2 n-6 and C20:3 n-6.
[c]Includes C18:3 n-3, C18:4 n-3, C20:4 n-3 and C21:5 n-3.

and the sum index, which are shown in Figure 3. The dietary regime did not influence any of the measured welfare parameters ($P > 0.05$). Temperature ($P = 0.026$) and CF (0.0005) affected the scoring for emaciation, with fish with low CF and fish reared at 6°C showing higher prevalence and severity of emaciation (Figure 3a). Moreover, scale loss was affected by temperature ($P = 0.033$) and CF ($P < 0.0001$; Figure 3b). Here, more scale loss was found in fish with low CF and reared at 6°C. The overall sum score index was significantly affected by the interaction of temperature and CF ($P = 0.039$) and by the main effect of CF ($P = 0.0004$; Figure 3c). Fish with low CF showed higher signs of welfare issues compared to those with high CF. This difference was particularly pronounced in fish reared at 15°C, whereas differences in welfare between high and low CFs in fish reared at 6°C were more balanced. No tank effect was found on any of the measured welfare parameters ($P > 0.05$).

3.3.4 | Gene expression

The relative expression of analysed genes in the liver tissue sampled at the end of the temperature trial is shown in Figure 4. Overall, there were no significant effects of CF and any of the tested interactions for the analysed genes ($P > 0.05$). The dietary regime affected only the relative levels of *fasn* ($P = 0.023$, $R^2 = 0.100$), with fish fed

TABLE 4 Survival during the temperature trial and means and S.D. of the traits recorded at the end of the temperature trial of ballan wrasse (*Labrus bergylta*) with high and low CF fed a standard-EPA or a high-EPA diet and reared at 15 or 6°C

	15°C						6°C					
	Standard EPA				High EPA				Standard EPA				High EPA			
	High CF		Low CF		High CF		Low CF		High CF		Low CF		High CF		Low CF	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Survival (%)	97.1	2.9	88.3	3.4	98.1	3.2	93.8	6.5	92.5	4.2	87.8	9.6	93.3	5.9	85.5	11.9
Body weight (g)	87.23	32.09	65.07	23.75	89.88	28.60	71.41	25.01	65.59	19.27	51.20	15.94	65.21	18.12	49.16	15.70
Standard length (mm)	152	19	140	17	153	18	145	18	142	15	134	14	142	14	133	14
SGR	0.151	0.146	0.178	0.157	0.163	0.113	0.17	0.155	−0.053	0.045	−0.061	0.065	−0.061	0.048	−0.062	0.053
TGC	2.435	0.393	2.23	0.345	2.476	0.324	2.292	0.365	−0.162	0.089	−0.162	0.114	−0.175	0.09	−0.162	0.089
HSI (%)	1.49	0.41	1.51	0.51	1.37	0.38	1.43	0.51	1.86	0.47	1.48	0.51	1.50	0.37	1.50	0.46
IFI (%)	0.98	0.69	0.62	0.51	1.18	0.62	0.96	0.56	0.34	0.32	0.15	0.21	0.25	0.29	0.14	0.18

Abbreviations: CF, condition factor; EPA, eicosapentaenoic acid; HSI, hepato-somatic index; IFI, intestinal fat index; SGR, specific growth rate; TGC, thermal growth coefficient.

standard-EPA diet showing higher relative expression levels. Temperature regime had a significant effect on the relative expression levels of *il-6* ($P = 0.015$; $R^2 = 0.258$; Figure 4b), *mstn* ($P = 0.001$; $R^2 = 0.458$; Figure 4e), *ghra* ($P = 0.02$; $R^2 = 0.158$; Figure 4f), *cpt1* ($P = 0.027$; $R^2 = 0.201$; Figure 4h) and *elovl5* ($P = 0.003$; $R^2 = 0.481$; Figure 4j). Although *ghra* was upregulated in fish reared at 15°C, the remaining genes with a significant effect of temperature were upregulated in fish reared at 6°C. Moreover, there was a tendency for higher expression levels of *hsp70* in fish reared at 6°C, which was, however, not significant due to high individual variation ($P > 0.05$). No differences were found for relative expression levels of *igf 1*, *igf 2* and *acox1* ($P > 0.05$; Figure 4c,d,i).

3.3.5 | Histology

The skin of ballan wrasse showed the typical features found in the skin of other fish species, including the epidermis with mucous cells and the dermis with scales. The results of the histological analyses are shown in Figure 5. CF did not significantly affect any of the skin histology parameters ($P > 0.05$), except the ratio of light/dark blue cells in the inner epidermal layer. Temperature significantly affected the number of mucous cells in the outer ($P < 0.0001$, $R^2 = 0.506$) and inner epidermal layers ($P = 0.0157$, $R^2 = 0.159$; Figure 5c). In both epidermal layers, fish reared at 15°C had more mucous cells compared to those reared at 6°C. The ratio between dark and light blue cells in the outer epidermal layer did not show any differences, whereas the ratio in the inner epidermal layer was slightly affected by temperature ($P = 0.018$, $R^2 = 0.211$) and CF ($P = 0.039$, $R^2 = 0.211$; Figure 5d). Temperature further affected the thickness of the epidermis in the outer layer ($P = 0.018$, $R^2 = 0.211$), with fish being reared at 15°C having a thicker epidermis (Figure 5e). Nonetheless, there was also a significant relationship between body weight and the thickness of the epidermis in the outer layer within both temperature treatments. Here, the relationship was best explained by a positive linear regression for fish reared at 15°C ($Y = 30.30 + 0.19x$; $P = 0.008$; $R^2 = 0.247$) and at 6°C ($Y = 18.69 + 0.33x$; $P = 0.023$; $R^2 = 0.363$). The epidermis thickness in the inner layer did not differ among treatments ($P > 0.05$). The ratio between mucous cells in the uttermost part of the epidermis was counted, and the ratio between these cells to the total number of mucous cells was measured. This ratio was significantly higher in fish reared at 6°C compared to those reared at 15°C ($P < 0.0001$, $R^2 = 0.515$; Figure 5f).

4 | DISCUSSION

4.1 | Feeding trial

Overall, the survival and performance of ballan wrasse during the feeding trial were good. The average CF was 2.7 at the end of the feeding trial, which was lower than that observed in smaller ballan wrasse (11 g) with CF of *c.* 3 (Kousoulaki *et al.*, 2021) but higher than that reported for larger fish (*c.* 700 g) with CF of *c.* 1.7 (Hamre *et al.*, 2013). The

Trait	Temperature	Diet	CF	Significant interactions	R^2
Survival	0.592	0.023	1.626***	–	0.044
Body weight	14.25***	0.08	10.61***	–	0.263
Standard length	8.13**	0.13	7.09***	–	0.153
SGR	51.22***	0.00	0.05	–	0.515
TGC	93.41***	0.01	0.13***	Temperature × CF (0.15***)	0.959
HSI	2.01	2.14*	0.68	–	0.080
IFI	35.85***	0.83	3.50***	Temperature × diet (1.79**)	0.424

Abbreviations: CF, condition factor; HSI, hepato-somatic index; IFI, intestinal fat index; SGR, specific growth rate; TGC, thermal growth coefficient.
*P < 0.05. **P < 0.01. ***P < 0.001.

TABLE 5 The proportion of total variation in each of the studied traits of ballan wrasse (*Labrus bergylta*) with high and low CF fed a standard-EPA or a high-EPA diet and reared at 15 or 6°C explained by each fixed effect and their interaction in the applied statistical model 3 with significance *P*-values and the R^2 values of the model

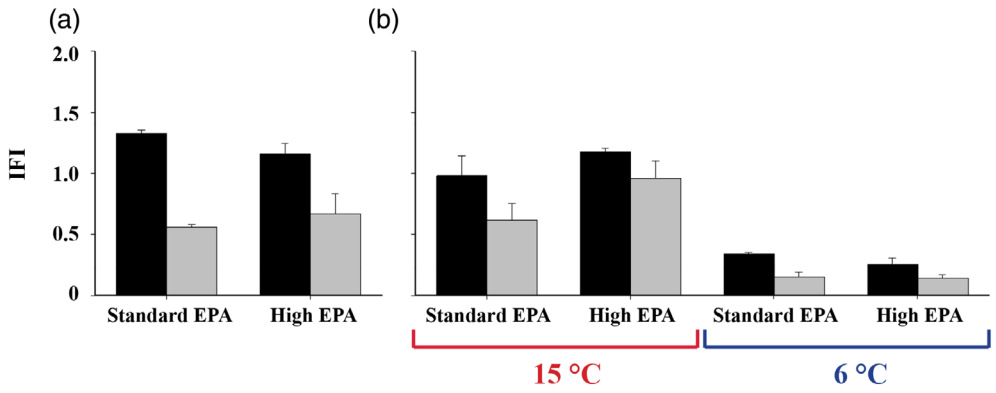

FIGURE 2 (a) Intestinal fat index (IFI) for ballan wrasse fed standard-eicosapentaenoic acid (EPA) diet and with high or low condition factor (CF) during the feeding trial. (b) IFI for ballan wrasse (*Labrus bergylta*) reared at 15 and 6°C in the following temperature trial. Values for bar plots represent means (±S.E.) among each treatment. ■, High CF; ■, Low CF.

dietary regime did not affect any of the measured parameters in terms of survival, weight, CF, HSI, IFI or chemical composition. This indicates that PUFA levels in standard-EPA diet were sufficient to support growth and performance under normal conditions. This was expected as the enhanced levels of PUFA in one diet during the feeding trial were meant to prepare the fish for a more stressful environment at a lower temperature during the following temperature trial. In other species, like salmon, it has been shown that the requirements for EPA and DHA increase under stressful conditions (Bou *et al*., 2017).

The FA composition in the WB at the end of the feeding trial reflected the FA levels in the two diets. As expected, the relative amount of EPA had increased in fish fed the diet supplemented with the EPA-rich EPAX oil concentrate, whereas the relative amount of the SFAs and MUFAs decreased. Finally, fish fed high-PUFA diet showed a tendency towards lower occurrence of scale loss compared to those fed standard-EPA diet. This may be related to the n-3 FAs in the diet that can have a positive effect on skin quality, as previously shown for salmon (Berge, unpubl. results).

4.2 | Temperature trial

4.2.1 | Performance and chemical and FA composition

Survival during the temperature trial was high, and no large mortality outbreaks occurred in any of the treatments. There was, however, higher mortality in one tank, which gave a significant tank effect in the model, which explained very little of the variation in survival and therefore needs to be considered carefully. Nonetheless, there was an effect of CF, and fish with higher CF had higher survival rates. There was no enhanced mortality in the cold treatment in the current experiment, contrasting the findings for ballan wrasse in sea cages at low temperatures (Geitung *et al*., 2020). Nonetheless, there were distinct differences in growth and performance between the temperature regimes. Positive SGR and TGC were observed in the 15°C treatment, albeit being lower than those observed in literature (Cavrois-Rogacki *et al*., 2019; Kousoulaki *et al*., 2021). Nevertheless, literature data on ballan wrasse growth are, to a large extent, restricted to earlier life stages and smaller body sizes (*e.g*., larval and weaning phases), where the growth rates are most often higher than those of larger fish. Fish reared at 6°C showed negative SGR and TGC, leading to weight loss over time. These results are comparable to those of Geitung *et al*. (2020), who found no growth in ballan wrasse during the winter season in sea cages. In the current study, fish with high CF were growing better, in particular, at 15°C. Together with improved survival, it appears that fish with high CF were coping better with low temperatures in this experiment. Yuen *et al*. (2019) revealed that ballan wrasse showed very low swimming activity and lower metabolic rates under cold temperatures. This metabolic depression or dormancy is commonly found in fish as an active reduction of energy costs when exposed to low temperatures (Crawshaw, 1984). This winter dormancy has been found and studied on a related labrid species, the cunner (*Tautogolabrus adspersus*), a north temperate species (Gerber

TABLE 6 Means and S.D. of the chemical composition of whole fish samples of ballan wrasse (*Labrus bergylta*) with high or low CF fed a standard-EPA or a high-EPA diet and reared at 15 or 6°C

	15°C						6°C					
	Standard EPA				High EPA				Standard EPA				High EPA			
	High CF		Low CF		High CF		Low CF		High CF		Low CF		High CF		Low CF	
Trait	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Dry matter (%)	30.17	0.75	28.64	1.00	29.58	0.23	28.00	1.10	25.94	0.95	24.40	0.39	25.88	0.46	23.92	0.59
Ash (%)	4.58	0.25	4.78	0.12	4.50	0.05	4.90	0.22	4.81	0.12	4.98	0.06	4.87	0.05	5.44	0.31
Fat (%)	7.91	0.73	6.52	1.10	7.26	0.51	5.99	1.21	4.54	0.77	3.28	0.34	4.86	0.36	2.82	0.67
Crude protein (N × 6.25) (%)	17.7	0.09	17.18	0.29	17.51	0.25	17.23	0.27	16.85	0.17	15.82	0.38	16.05	0.34	15.87	0.20
Energy (MJ kg^{-1})	7.19	0.38	6.62	0.35	7.02	0.22	6.32	0.52	5.39	0.52	4.55	0.09	5.40	0.14	4.28	0.38

Abbreviations: CF, condition factor; EPA, eicosapentaenoic acid.

et al., 2022; Lewis & Driedzic, 2007; Speers-Roesch *et al.*, 2018). Observations from this study, where fish reared at 6°C were inactive and often found lying on the bottom of the tank (pers. obs.), indicate that a similar winter dormancy might exist in ballan wrasse.

The HSI as lipid storage may serve as an indicator for energy reserves in fish and has been shown to increase with higher temperatures in coral reef fish (Bernal *et al.*, 2018). The HSI of fish in the current experiment was lower than that previously found for ballan wrasse of different sizes (Hamre *et al.*, 2013; Kousoulaki *et al.*, 2021) but did not differ among temperature treatments. Intestinal fat seems to be an important storage for energy in ballan wrasse. Fish with low CF also had fewer intestinal fat reserves at the start of the temperature trial. At the end of the temperature trial, the IFI was somewhat reduced in fish with initially high CF and IFI but was very low in fish with initially low CF and IFI.

The better state of fish reared at high temperatures and with high CF was also confirmed by the chemical composition. Higher amounts of fat, crude protein and energy as well as lower ash content were found in fish reared at 15°C and to a lower extent but still significant for fish with higher CF.

Interestingly, the dietary regime from the feeding trial did not have any effect on the performance of the fish during the temperature trial. Nonetheless, the FA profile at the end of the temperature trial remained to be largely influenced by the previous dietary treatment and reflected FA levels in the diets. Moreover, the temperature had a dominant effect on some of the FAs. Although fish reared at 15°C had higher levels of fat (SFA) at the end of the temperature trial, n-6 PUFAs were used up to a larger extent in fish reared at 15°C than at 6°C. This is in line with results for gilthead seabream (Torno *et al.*, 2018), milkfish and grass carp (Hsieh & Kuo, 2005), where higher SFA levels and lower PUFA levels were found for fish reared at higher temperatures. CF, on the contrary, did not have a strong effect on the FA profile of the fish.

4.2.2 | Welfare scoring

When considering the future use of cleaner fish, health and welfare aspects are of high importance, and currently increasing concerns are raised on the welfare of cleaner fish in salmon cages (Brooker *et al.*, 2018; Mo & Poppe, 2018; Powell *et al.*, 2018). So-called operational welfare indicators that were previously developed for ballan wrasse (Espmark *et al.*, 2019) were also used in the current study to evaluate the welfare of experimental ballan wrasse under simulated cold winter temperatures. Temperature and CF both significantly affected the welfare of ballan wrasse. Fish under cold temperatures and with low initial CF showed higher prevalence and severity of emaciation. This is in line with results showing negative growth rates and low energy reserves underlining the inferior condition of fish for these treatments compared to fish reared at 15°C and with high CF. Also, scale loss was affected by temperature and CF, and fish reared at 6°C and with low initial CF had more scale loss compared to those reared at 15°C and with high CF. This may be related to the behaviour of fish at

Trait	Temperature	Diet	CF	Significant interactions	R^2
Dry matter	78.75***	0.94	13.00***	–	0.9370
Ash	31.01**	5.36	30.70***	–	0.7960
Fat	69.43***	0.81	16.61***	–	0.884
Crude protein (N × 6.25)	71.60***	2.16	11.74**	–	0.8900
Energy	76.77***	0.70	14.14***	–	0.9210

TABLE 7 The proportion of the total variation in chemical composition of whole-body samples of ballan wrasse (*Labrus bergylta*) with high or low CF fed a standard-EPA or a high-EPA diet and reared at 15 or 6°C explained by each fixed effect in the applied statistical model 4 with significance *P*-value and the R^2 values of the model

Abbreviations: CF, condition factor; EPA, eicosapentaenoic acid.
**$P < 0.01$. ***$P < 0.001$.

TABLE 8 Means and S.D. of fatty acid composition (% of total fatty acids) of total lipids extracted from whole fish samples of ballan wrasse (*Labrus bergylta*) with high or low CF fed a standard-EPA or a high-EPA diet and reared at 15 or 6°C

	15°C								6°C							
	Standard EPA				High EPA				Standard EPA				High EPA			
	High CF		Low CF		High CF		Low CF		High CF		Low CF		High CF		Low CF	
Fatty acid	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
14:0	6	0.2	6.2	0.2	5.2	0.1	5.7	0.2	5	0.2	5.3	0.2	4.1	0.1	4.2	0.2
16:0	16.2	0.1	16	0.5	15	0.1	15.6	0.3	15.7	0.3	15.9	0.2	13.6	0.2	14.8	0.7
18:0	3.8	0.1	3.8	0.3	4	0.1	4.1	0.2	3.8	0.3	3.8	0.1	4.3	0.3	4.5	0.4
SFA	26.1	0.2	26.1	0.7	24.3	0	25.5	0.7	24.6	0.3	25.1	0.3	22.1	0.1	23.5	0.9
16:1 n-7	6.2	0.1	6.2	0.1	5.6	0.1	5.9	0.2	6.3	0.3	6.3	0.1	5.3	0.2	5.1	0.3
18:1	24.2	0.2	23.9	0.3	23.2	0.2	23.4	0.7	24.3	0.2	23.9	0.2	23.1	0.8	23.1	0.6
20:1	5.4	0.2	5.1	0.1	4	0.1	4.3	0.2	5.5	0.3	5.2	0.2	3.7	0.2	3.7	0.2
22:1	3.5	0.2	3.1	0.2	1.8	0.1	1.9	0.1	3.9	0.4	3.3	0.3	1.6	0.1	1.7	0
MUFA[a]	39.7	0.6	38.7	0.3	35	0.2	35.9	1.2	40.4	0.7	39.2	0.3	34.1	1.2	34	0.8
18:2 n-6	4.4	0.1	4.4	0.2	4.4	0.2	4.3	0.1	4.9	0.1	4.8	0.1	4.8	0.1	4.7	0.1
20:4 n-6 (ARA)	0.7	0	0.7	0.1	1.2	0.1	1	0.1	0.9	0.1	1	0.1	1.5	0.1	1.7	0.2
n-6 PUFA[b]	5.7	0.1	5.8	0.3	6.4	0.1	6	0.1	6.5	0.1	6.4	0.1	7.2	0.2	7.1	0.1
20:5 n-3 (EPA)	9	0.2	9.5	0.3	14.3	0.2	12.6	0.6	8.6	0.2	9.3	0.4	15.9	0.6	14.6	0.8
22:5 n-3	1.5	0	1.5	0.1	2	0.1	1.8	0.2	1.5	0.1	1.5	0.1	2.1	0.1	2.1	0.1
22:6 n-3 (DHA)	9.5	0.2	9.9	0.3	10	0.1	10	1.4	10.7	0.7	11.1	0.2	10.9	0.2	12	0.4
n-3 PUFA[c]	23.2	0.4	24.3	0.4	29.6	0.2	27.8	2	23.5	0.9	24.5	0.3	32	0.8	31.4	1.5

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
[a]Includes C24:1 n-9.
[b]Includes C20:2 n-6 and C20:3 n-6.
[c]Includes C18:3 n-3, C18:4 n-3, C20:4 n-3 and C21:5 n-3.

low temperatures that were seen to be inactive and often lying on the bottom of the tank, which may have led to increased scale loss. In a sea cage study that looked at the welfare of cleaner fish during cold winter temperatures, the measured welfare indicators improved throughout the experimental period (Geitung *et al.*, 2020). Nonetheless, this may be related to increased mortality of fish with particularly low welfare as mortality in the sea cage experiment was higher compared to that in the current study. Overall, the results of this study showed that the welfare of ballan wrasse was negatively impacted by cold winter temperatures. Nonetheless, the CF of the fish before entering a period with cold temperatures appeared to be important for the welfare.

In the present study, only two very different temperatures were investigated; therefore, more information is needed on which exact temperature between 15 and 6°C ballan wrasse become affected by the reduced temperatures. Future studies should also elucidate the duration of low-temperature periods that ballan wrasse can tolerate.

4.2.3 | Gene expression

Growth hormone (GH) is involved in many physiological processes, most importantly growth. GH and its receptors are known to be

TABLE 9 The proportion of the total variation in each fatty acid of whole fish samples of ballan wrasse (*Labrus bergylta*) explained by each fixed effect and their interaction in the applied statistical model with significance *P*-values and the R^2 value of Model 4

Fatty acid	Temperature	Diet	CF	Temperature × diet	Diet × CF	R^2
14:0	60.21***	31.26***	2.55**	1.58*	*P* > 0.05	0.962
16:0	16.22*	52.26***	6.61**	4.77*	7.12**	0.920
18:0	7.02	43.89***	3.44	*P* > 0.05	*P* > 0.05	0.591
SFA	38.72**	37.19***	8.29**	*P* > 0.05	4.05*	0.931
16:1 n-7	6.94*	68.33***	0.21	11.30***	*P* > 0.05	0.905
18:1	0.49	52.54***	0.95	*P* > 0.05	*P* > 0.05	0.634
20:1	0.73	88.83***	0.39	3.55**	2.38**	0.961
22:1	0.03	90.68***	1.23*	1.44*	3.09**	0.969
MUFA[a]	0.54	86.77***	0.49	3.74**	2.19*	0.948
18:2 n-6	80.57***	1.07	1.91	*P* > 0.05	*P* > 0.05	0.851
20:4 n-6 (ARA)	30.79***	55.32***	0.35	5.03**	2.36*	0.947
n-6 PUFA[b]	60.40***	29.60***	1.55*	1.55*	*P* > 0.05	0.955
20:5 n-3 (EPA)	1.83*	88.43***	0.71*	3.70***	3.58***	0.986
22:5 n-3	2.78	79.86***	1.12	6.63**	*P* > 0.05	0.944
22:6 n-3 (DHA)	53.28**	5.63	7.00	*P* > 0.05	*P* > 0.05	0.747
n-3 PUFA[c]	5.28*	82.56***	0.02	3.96**	2.59**	0.960

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
[a]Includes C24:1 n-9.
[b]Includes C20:2 n-6 and C20:3 n-6.
[c]Includes C18:3 n-3, C18:4 n-3, C20:4 n-3 and C21:5 n-3.
*P < 0.05. **P < 0.01. ***P < 0.0.01.

influenced by temperature, and their levels show a peak in warm summer months in several different fish species (reviewed in Deane & Woo, 2009). In the current study, fish reared at 6°C with negative SGR and TGC had downregulated *GHrα* levels compared to fish reared at 15°C, indicating that temperature mediated growth through the GH system (Gabillard *et al*., 2005). It will be interesting to additionally look at the plasma GH levels to confirm this in future studies. Moreover, *mstn* is known as a negative marker for muscle growth (McPherron *et al*., 1997) and was accordingly upregulated in fish reared at 6°C. Findings confirm the positive effect of higher temperature on growth markers by upregulating *GHrα* and downregulating *mstn*. Fish reared at 6°C further had upregulated levels of *il-6* compared to fish reared at 15°C and a tendency to higher levels of *hsp70*. The higher immune response and higher stress levels in fish at cold temperatures indicate the negative impact of low winter temperatures on the condition of ballan wrasse, whereas fish reared at 15°C appeared to be in a better condition. Suboptimal temperatures are known to negatively impact the immune system and increase stress in teleosts represented by increased expression levels of heat shock proteins (HSP) (reviewed in Abram *et al*., 2017; Roberts *et al*., 2010). In the current experiment, two genes involved in the synthesis and oxidation of FAs (*elovl5*, *cpt1*) were upregulated in fish reared at 6°C compared to those reared at 15°C. Upregulation of *cpt1* may indicate that fish were burning FAs to a higher extent under cold temperatures, and these results are in line with complementary findings in this study, showing that fish reared at cold temperatures used up most of their energy reserves during this trial. Increased gene expression of *elovl5* involved in PUFA syntheses may be explained by the need for PUFAs to maintain the fluidity of the cell membranes at low temperatures.

4.2.4 | Histology

To the best of the authors' knowledge, this is the first publication on skin histology analyses on ballan wrasse. There were several samples with damaged epidermis that had to be removed from the analyses, especially at low temperatures. This indicates that the sampling procedures need to be optimized for this species. The high number of damaged samples in the skin from the 6°C group may also indicate other biological effects caused by temperature, such as weaker attachment of the epidermis to the basement membrane, as previously observed in other fish species exposed to stress (Ytteborg *et al*., 2023). The epidermis was thicker in fish reared at 15°C compared to those at 6°C. This may be related to fish size, as fish were larger when reared at 15°C. This was supported by a significant positive relationship between body weight and epidermis thickness within both temperature treatments. Nonetheless, a decrease in epidermis thickness has also been related to stress in salmon (Jensen *et al*., 2015), carp (van der Marel *et al*., 2010) and rainbow trout (Iger *et al*., 1994). Moreover, fish reared at 15°C had a higher number of overall mucous cells in the inner and outer epidermis layers. The mucous in fish skin serves as an important biological barrier acting as a defence mechanism

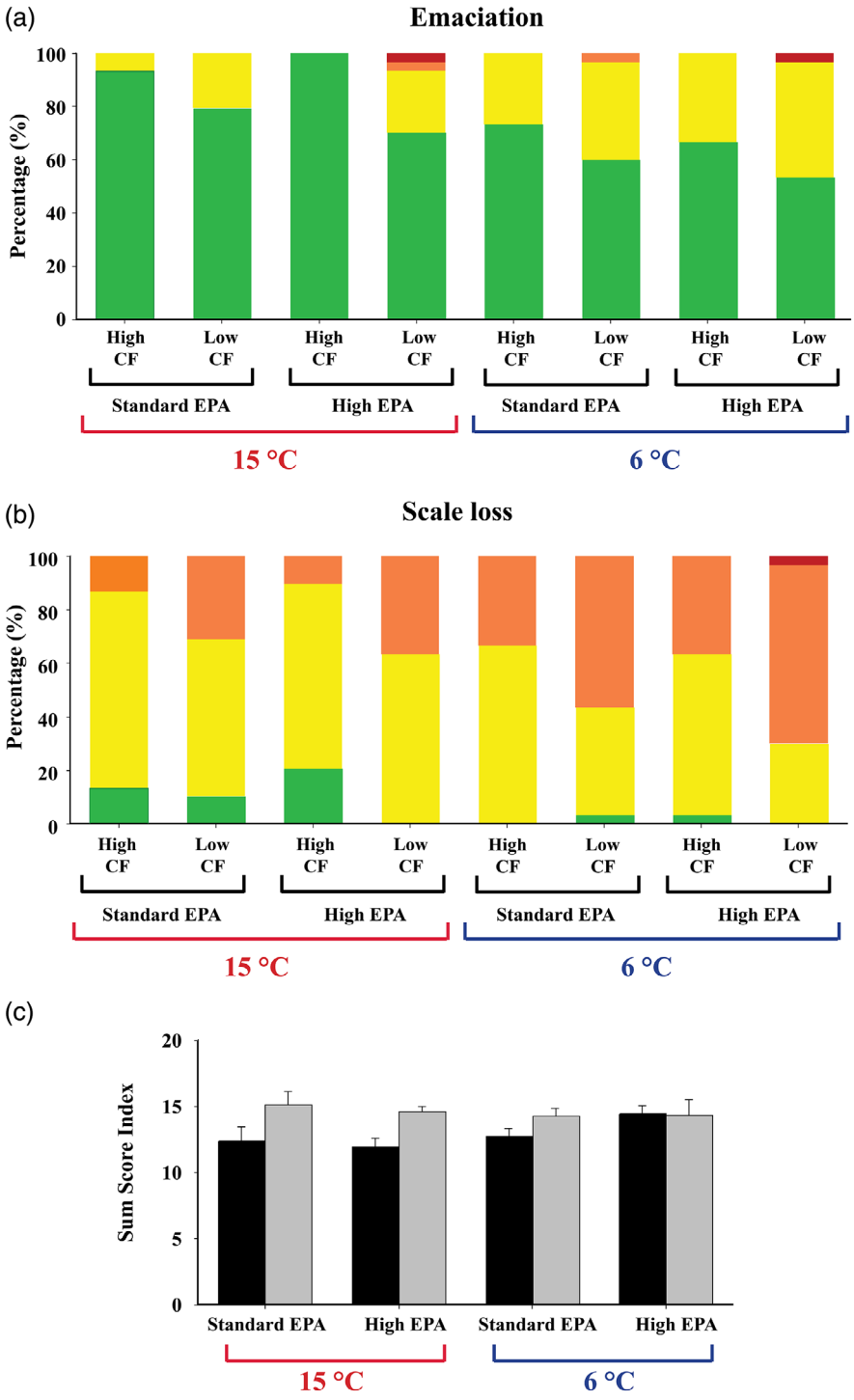

FIGURE 3 Welfare scoring of ballan wrasse (*Labrus bergylta*) with high or low CF fed standard-eicosapentaenoic acid (EPA) or high-EPA diet and reared at 15 or 6°C for (a) emaciation, (b) scale loss and (c) the sum score index (sum of all 12 measured welfare parameters). Values for bar plots represent means (±S.E.) among each treatment. Severity ■, 0; ■, 1; ■, 2; ■, 3; ■, High CF; ■, Low CF

(Esteban, 2012). Several studies have shown an increased number of mucous cells as a stress response in European sea bass (*Dicentrarchus labrax*) (Vatsos *et al*., 2010), Arctic charr (*Salvelinus alpinus*) (Christiansen *et al*., 1991), carp (Iger & Bonga, 1994) or cod (*Gadus morhua*) (Ytteborg *et al*., 2020). The mucous cell response may, however, be species- or stressor-specific. This study indicates that cold water temperature hampered the production of mucous cells in ballan wrasse. Besides the overall number of mucous cells, their organization in the epidermal layers may serve as a stress indicator. In this study, the ratio between mucous cells in the outer part of the epidermis to the overall number of mucous cells was higher in fish reared at 6°C than that at 15°C. This organization of mucous cells has been related to increased stress in other species such as salmon (Jensen *et al*., 2015; Sveen *et al*., 2016), cod (Ytteborg *et al*., 2020), lumpfish (Ytteborg *et al*., 2023) and sea bass (Vatsos *et al*., 2010).

Also, the ratio of light blue to dark blue cells was higher in fish reared at 6°C compared to those reared at 15°C in the inner part of the epidermis. Although the coloration of the mucous cells is due to

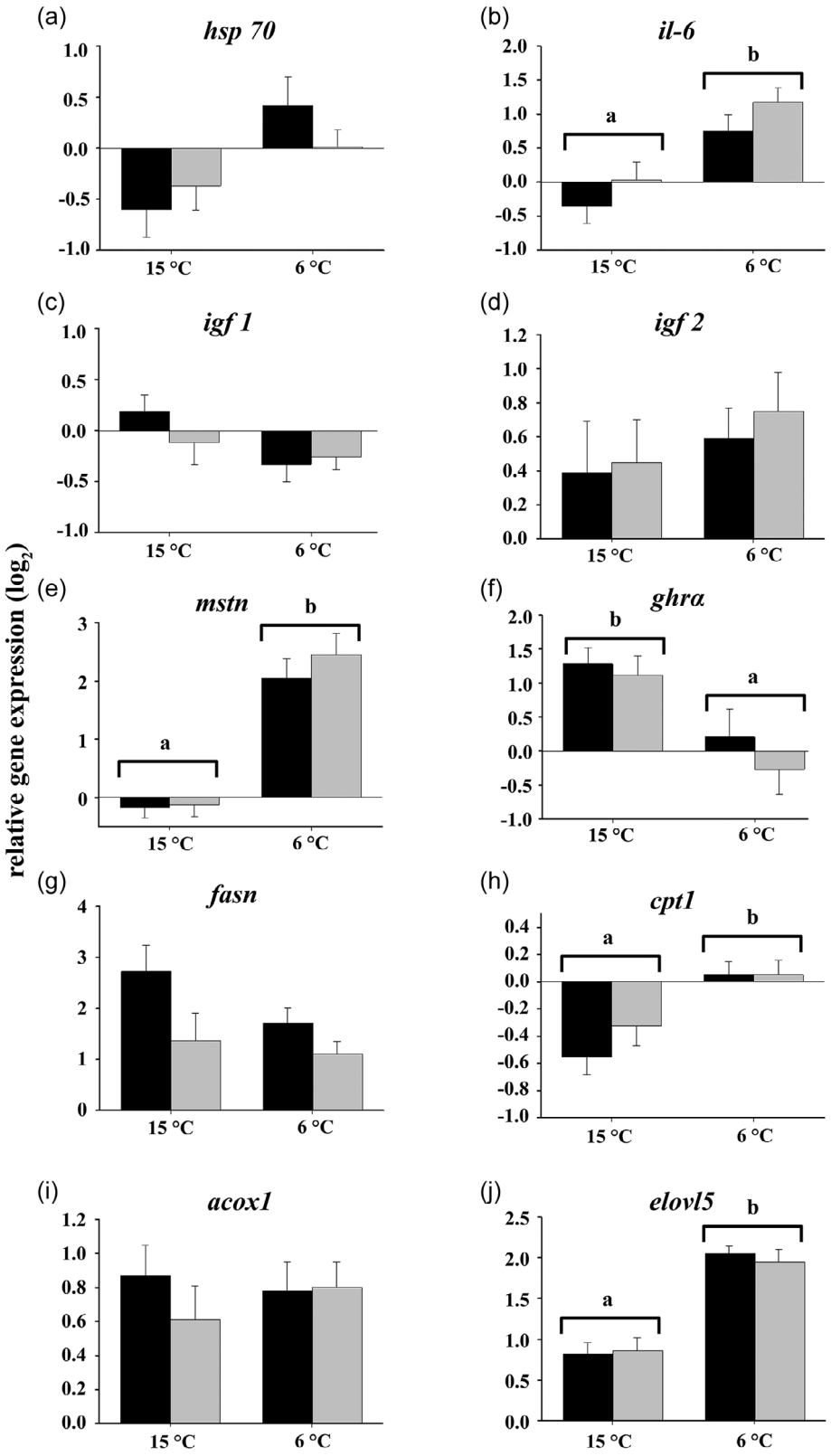

FIGURE 4 Relative gene expression levels (log_2) in liver samples of ballan wrasse (*Labrus bergylta*) fed standard-eicosapentaenoic acid (EPA) or high-EPA diet reared at 15 and 6°C for (a) *hsp70*, (b) *il-6*, (c) *igf1*, (d) *igf2*, (e) *mstn*, (f) *ghrα*, (g) *fasn*, (h) cpt1, (i) *acox1* and (j) *elovl5*. Values for bar plots represent means (±S.E.) among each treatment. Different lowercase letters represent a significant statistical difference for the effect of temperature ($P < 0.05$). ■, Standard EPA; ■, High EPA

the pH value of the mucous produced and caused by glycosylation of mucins, the function of the differently coloured mucous cell is not yet known and requires further study. Overall, fish reared at 15°C appeared to have better skin quality, whereas skin from fish reared at 6°C showed signs of stress based on the experience from other species. Nonetheless, more studies on ballan wrasse skin are needed to confirm these features as stress indicators in this species, allowing a comparison to other species.

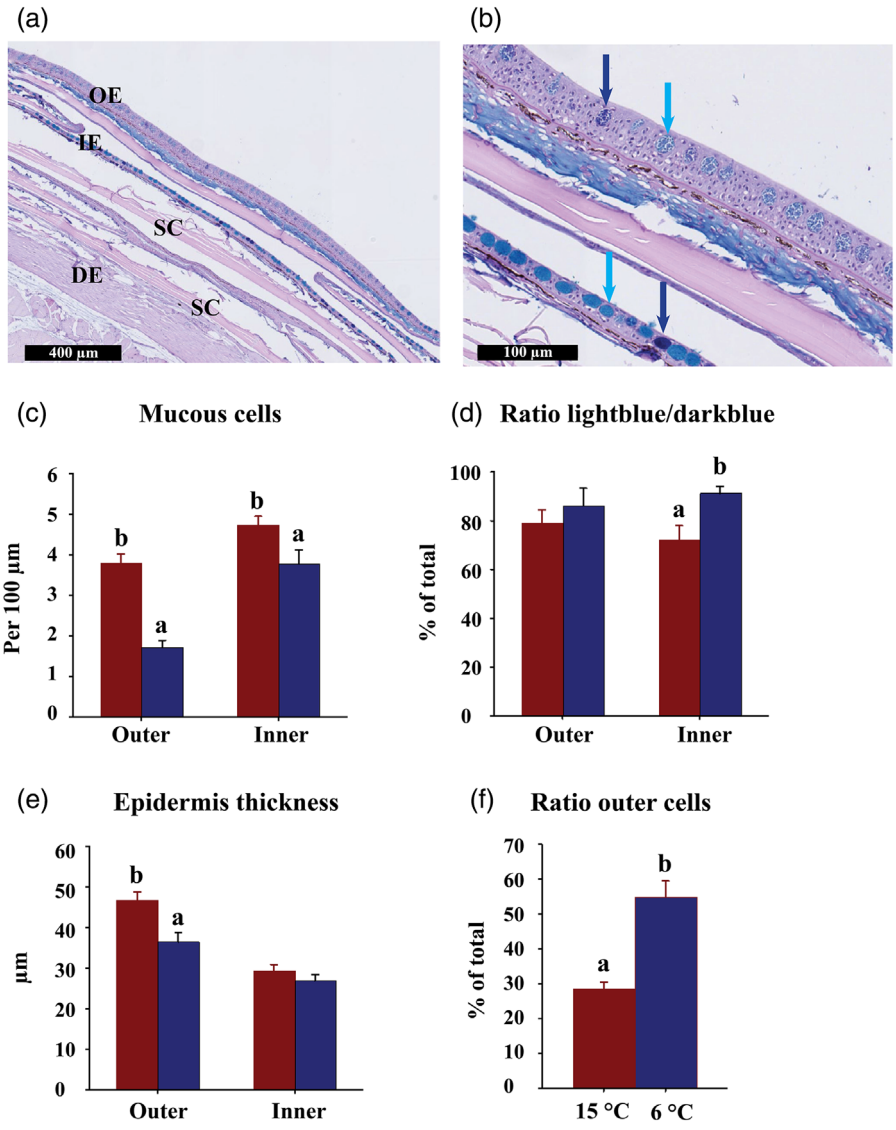

FIGURE 5 Histological evaluation of ballan wrasse skin. (a) Overview of ballan wrasse (*Labrus bergylta*) skin showing the outer epidermis (OE), inner epidermis (IE), scales (SC), dermis (DE). (b) Increased magnification of the epidermis showing different coloured mucous cells in the inner and outer epidermis (light blue and dark blue arrows). (c) The number of mucous cells in the outer and inner epidermal layers. (d) The ratio between light blue and dark blue cells in the outer and inner epidermal layers. (e) Epidermis thickness in the outer and inner epidermal layers. (f) The ratio between mucous cells in the uttermost part of the epidermis and total mucous cells. Values for bar plots represent means (±S.E.) among each treatment. Different lowercase letters represent a significant statistical difference for the effect of temperature ($P < 0.05$). ■, 15°C; ■, 6°C

5 | CONCLUSIONS

Low simulated winter temperatures had a distinct impact on the performance and welfare of ballan wrasse. Although the dietary treatment solely affected the FA profiles but not the performance during different temperatures, there were clear indications of the importance of fish condition. High CF is important for ballan wrasse when they need to endure cold temperatures during winter, which is a clear stressor for this species. High temperatures resulted in higher growth, higher fat and energy reserves and less ash. Fish reared at lower temperatures, on the contrary, showed negative growth rates, burned higher amounts of FAs and had very little energy reserves left at the end of the temperature trial. It can be speculated that this would affect the delousing efficacy of ballan wrasse during cold winter temperatures, but further studies are needed to quantify their cleaning abilities. Moreover, low temperature impacted the welfare of the fish measured in terms of external welfare scoring, gene expression and skin histology analyses. This should be considered and implemented in the industry when using ballan wrasse in salmon sea cages during winter months in the future. The current tendency to select cleaner fish species depending on the season is supported by the outcome of this study. Promising results demonstrated that fish in better condition coped better with low temperatures by showing better survival, growth, performance and welfare. Future studies should focus on defining parameters that can improve the condition and welfare of ballan wrasse before deployment and during different seasonal challenges.

AUTHOR CONTRIBUTIONS

Ideas (J.S.K., G.M.B., K.K., T.K.Ø., E.Y., I.L.); data generation (J.S.K., G.M.B., I.L.); data analysis (J.S.K., G.M.B., T.K.Ø., E.Y., I.L., B.G.); manuscript preparation (J.S.K., G.M.B., K.K., T.K.Ø., E.Y., B.G., I.L.); and funding (I.L.).

ACKNOWLEDGEMENTS

The authors acknowledge the contributions of the laboratory collaborators from Nofima AS BioLab and Aqualab, the staff of the Feed Technology Center of Nofima in Bergen for the production of the experimental diets and the Nofima Aquaculture Research Center Sunndalsøra for the execution of the experimental trials. Special thanks to Karoline Valseth and Rita Storslett, who took part in the experimental work, as well as Vibeke Voldvik, Anders Gjeldnes and Kristin Skei Nerdal, who assisted with laboratory analyses.

ORCID

Johanna S. Kottmann https://orcid.org/0000-0002-4928-6471

REFERENCES

Abram, Q. H., Dixon, B., & Katzenback, B. A. (2017). Impacts of low temperature on the teleost immune system. *Biology*, *6*(4), 39. https://doi.org/10.3390/biology6040039.

Barrett, L. T., Oppedal, F., Robinson, N., & Dempster, T. (2020). Prevention not cure: A review of methods to avoid sea lice infestations in salmon aquaculture. *Reviews in Aquaculture*, *12*(4), 2527–2543. https://doi.org/10.1111/raq.12456.

Bernal, M. A., Donelson, J. M., Veilleux, H. D., Ryu, T., Munday, P. L., & Ravasi, T. (2018). Phenotypic and molecular consequences of stepwise temperature increase across generations in a coral reef fish. *Molecular Ecology*, *27*(22), 4516–4528. https://doi.org/10.1111/mec.14884.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. https://doi.org/10.1139/o59-099.

Bou, M., Berge, G. M., Baeverfjord, G., Sigholt, T., Østbye, T. K., & Ruyter, B. (2017). Low levels of very-long-chain n-3 PUFA in Atlantic salmon (Salmo salar) diet reduce fish robustness under challenging conditions in sea cages. *Journal of Nutritional Science*, *6*, e32. https://doi.org/10.1017/jns.2017.28.

Bowden, T. J., Thompson, K. D., Morgan, A. L., Gratacap, R. M. L., & Nikoskelainen, S. (2007). Seasonal variation and the immune response: A fish perspective. *Fish & Shellfish Immunology*, *22*(6), 695–706. https://doi.org/10.1016/j.fsi.2006.08.016.

Brooker, A. J., Papadopoulou, A., Gutiérrez, C., Rey, S., Davie, A., & Migaud, H. (2018). Sustainable production and use of cleaner fish for the biological control of sea lice: Recent advances and current challenges. *The Veterinary Record*, *183*(12), 383. https://doi.org/10.1136/vr.104966.

Cavrois-Rogacki, T., Davie, A., Monroig, O., & Migaud, H. (2019). Elevated temperature promotes growth and feed efficiency of farmed ballan wrasse juveniles (*Labrus bergylta*). *Aquaculture*, *511*, 734237. https://doi.org/10.1016/j.aquaculture.2019.734237.

Cavrois-Rogacki, T., Drabikova, L., Migaud, H., & Davie, A. (2021). Deformities prevalence in farmed ballan wrasse (*Labrus bergylta*) in relation to hatchery origin and life stage. *Aquaculture*, *533*, 736212. https://doi.org/10.1016/j.aquaculture.2020.736212.

Christiansen, J. S., Jobling, M., Amin, A. B., & Ribe, H. (1991). Histomorphological changes in epidermal structure of juvenile Arctic charr (*Salvelinus alpinus* L.) subjected to sustained exercise. *Canadian Journal of Zoology*, *69*(9), 2450–2455. https://doi.org/10.1139/z91-344.

Costello, M. J. (2009). The global economic cost of sea lice to the salmonid farming industry. *Journal of Fish Diseases*, *32*(1), 115–118. https://doi.org/10.1111/j.1365-2761.2008.01011.x.

Crawshaw, L. I. (1984). Low-temperature dormancy in fish. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *246*(4), R479–R486. https://doi.org/10.1152/ajpregu.1984.246.4.r479

Deane, E. E., & Woo, N. Y. S. (2009). Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: A review. *Reviews in Fish Biology and Fisheries*, *19*, 97–120. https://doi.org/10.1007/s11160-008-9091-0.

Espmark, Å. M., Noble, C., Kolarevic, J., Berge, G. M., Aas, G. H., Tuene, S., … Gjerde, B. (2019). Velferd hos rensefisk – operative velferdsindikatorer (OVI), Nofima rapport 12/2019.

Esteban, M. Á. (2012). An overview of the immunological defenses in fish skin. *International Scholarly Research Notices*, *2012*, 1–29. https://doi.org/10.5402/2012/853470.

Fjelldal, P. G., Madaro, A., Hvas, M., Stien, L. H., Oppedal, F., & Fraser, T. W. K. (2020). Skeletal deformities in wild and farmed cleaner fish species used in Atlantic salmon *Salmo salar* aquaculture. *Journal of Fish Biology*, *98*(4), 1049–1058. https://doi.org/10.1111/jfb.14337.

Gabillard, J. C., Weil, C., Rescan, P. Y., Navarro, I., Gutierrez, J., & Le Bail, P. Y. (2005). Does the GH/IGF system mediate the effect of water temperature on fish growth? A review. *Cybium*, *29*(2), 107–117.

Geitung, L., Wright, D. W., Oppedal, F., Stien, L. H., Vågseth, T., & Madaro, A. (2020). Cleaner fish growth, welfare and survival in Atlantic salmon sea cages during an autumn-winter production. *Aquaculture*, *528*, 735623. https://doi.org/10.1016/j.aquaculture.2020.735623.

Gerber, L., MacSween, C. E., Staples, J. F., & Gamperl, A. K. (2022). Cold-induced metabolic depression in cunner (*Tautogolabrus adspersus*): A multifaceted cellular event. *PLoS One*, *17*(8), e0271086. https://doi.org/10.1371/journal.pone.0271086.

Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biological Reviews of the Cambridge Philosophical Society*, *79*(2), 409–427. https://doi.org/10.1017/S1464793103006328.

Hamre, K., Nordgreen, A., Grøtan, E., & Breck, O. (2013). A holistic approach to development of diets for ballan wrasse (*Labrus berggylta*) – a new species in aquaculture. *PeerJ*, *1*, e99. https://doi.org/10.7717/peerj.99.

Hazel, J. R. (1979). Influence of thermal acclimation on membrane lipid composition of rainbow trout liver. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *236*(1), R91–R101. https://doi.org/10.1152/ajpregu.1979.236.1.r91.

Hsieh, S. L., & Kuo, C. M. (2005). Stearoyl-CoA desaturase expression and fatty acid composition in milkfish (*Chanos chanos*) and grass carp (*Ctenopharyngodon idella*) during cold acclimation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *141*(1), 95–101. https://doi.org/10.1016/j.cbpc.2005.02.001.

Hvas, M., Folkedal, O., Imsland, A., & Oppedal, F. (2018). Metabolic rates, swimming capabilities, thermal niche and stress response of the lumpfish, *Cyclopterus lumpus*. *Biology Open*, *7*(9), bio036079. https://doi.org/10.1242/bio.036079.

Iger, Y., & Bonga, S. E. V. (1994). Cellular responses of the skin of carp (*Cyprinus carpio*) exposed to acidified water. *Cell and Tissue Research*, *275*, 481–492. https://doi.org/10.1007/BF00318817.

Iger, Y., Jenner, H. A., & Bonga, S. E. W. (1994). Cellular responses in the skin of the trout (*Oncorhynchus mykiss*) exposed to temperature elevation. *Journal of Fish Biology*, *44*(6), 921–935. https://doi.org/10.1111/j.1095-8649.1994.tb01265.x.

Jensen, L. B., Boltana, S., Obach, A., McGurk, C., Waagbø, R., & Mackenzie, S. (2015). Investigating the underlying mechanisms of temperature-related skin diseases in Atlantic salmon, *Salmo salar* L., as measured by quantitative histology, skin transcriptomics and composition. *Journal of Fish Diseases*, *38*(11), 977–992. https://doi.org/10.1111/jfd.12314.

Johnston, I. A., & Dunn, J. (1987). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. *Symposia of the Society for Experimental Biology*, *41*, 67–93.

Kabeya, N., Yevzelman, S., Oboh, A., Tocher, D. R., & Monroig, O. (2018). Essential fatty acid metabolism and requirements of the cleaner fish, ballan wrasse *Labrus bergylta*: Defining pathways of long-chain

polyunsaturated fatty acid biosynthesis. *Aquaculture*, *488*, 199–206. https://doi.org/10.1016/j.aquaculture.2018.01.039.

Kousoulaki, K., Bogevik, A. S., Skiftesvik, A. B., Jensen, P. A., & Opstad, I. (2015). Marine raw material choice, quality and weaning performance of Ballan wrasse (*Labrus bergylta*) larvae. *Aquaculture Nutrition*, *21*(5), 644–654. https://doi.org/10.1111/anu.12186.

Kousoulaki, K., Grøtan, E., Kortner, T. M., Berge, G. M., Haustveit, G., Krogdahl, Å., … Lein, I. (2021). Technical feed quality influences health, digestion patterns, body mineralization and bone development in farming of the stomachless cleaner fish ballan wrasse (*Labrus bergylta*). *Animal Feed Science and Technology*, *274*, 114830. https://doi.org/10.1016/j.anifeedsci.2021.114830.

Lewis, J. M., & Driedzic, W. R. (2007). Tissue-specific changes in protein synthesis associated with seasonal metabolic depression and recovery in the north temperate labrid, *Tautogolabrus adspersus*. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *293*(1), R474–R481. https://doi.org/10.1152/ajpregu.00594.2006.

Malek, R. L., Sajadi, H., Abraham, J., Grundy, M. A., & Gerhard, G. S. (2004). The effects of temperature reduction on gene expression and oxidative stress in skeletal muscle from adult zebrafish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *138*(3), 363–373. https://doi.org/10.1016/j.cca.2004.08.014.

McPherron, A., Lawler, A. M., & Lee, S. J. (1997). Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature*, *387*(6628), 83–90. https://doi.org/10.1038/387083a0.

Mo, T. A., & Poppe, T. T. (2018). Risiko ved bruk av rensefisk i fiskeoppdrett. *Norsk Veterinærtidsskrift*, *130*, 90–92.

Morel, G. M., Shrives, J., Bossy, S. F., & Meyer, C. G. (2013). Residency and behavioural rhythmicity of ballan wrasse (*Labrus bergylta*) and rays (*Raja* spp.) captured in Portelet Bay, Jersey: Implications for marine protected area design. *Journal of the Marine Biological Association of the United Kingdom*, *93*(5), 1407–1414. https://doi.org/10.1017/S0025315412001725.

Noble, C., Iversen, M. H., Lein, I., Kolarevic, J., Johansen, L., Berge, G. M., … Espmark, Å. M. (2019). RENSVEL OWI FACT SHEET SERIES : An introduction to Operational and Laboratory-based Welfare Indicators for lumpfish (*Cyclopterus lumpus* L .), pp. 46.

Norwegian Directorate of Fisheries. (2021). Cleanerfish (Lumpfish and Wrasse). https://www.fiskeridir.no/English/Aquaculture.

Overton, K., Barrett, L. T., Oppedal, F., Kristiansen, T. S., & Dempster, T. (2020). Sea lice removal by cleaner fish in salmon aquaculture: A review of the evidence base. *Aquaculture Environment Interactions*, *12*, 31–44. https://doi.org/10.3354/aei00345.

Pfaffl, M. W. (2004). Quantification strategies in real-time PCR. In S. A. Bustin (Ed.), *AZ of quantitative PCR* (pp. 89–113). La Jolla, CA: IUL Biotechnology Series, International University Line.

Powell, A., Treasurer, J. W., Pooley, C. L., Keay, A. J., Lloyd, R., Imsland, A. K., & Garcia de Leaniz, C. (2018). Use of lumpfish for sea-lice control in salmon farming: Challenges and opportunities. *Reviews in Aquaculture*, *10*(3), 683–702. https://doi.org/10.1111/raq.12194.

Roberts, R. J., Agius, C., Saliba, C., Bossier, P., & Sung, Y. Y. (2010). Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: A review. *Journal of Fish Diseases*, *33*(10), 789–801. https://doi.org/10.1111/j.1365-2761.2010.01183.x.

Speers-Roesch, B., Norin, T., & Driedzic, W. R. (2018). The benefit of being still: Energy savings during winter dormancy in fish come from inactivity and the cold, not from metabolic rate depression. *Proceedings of the Royal Society B*, *285*(1886), 20181593. https://doi.org/10.1098/rspb.2018.1593.

Sveen, L. R., Timmerhaus, G., Torgersen, J. S., Ytteborg, E., Jørgensen, S. M., Handeland, S., … Takle, H. (2016). Impact of fish density and specific water flow on skin properties in Atlantic salmon (*Salmo salar* L.) post-smolts. *Aquaculture*, *464*, 629–637. https://doi.org/10.1016/j.aquaculture.2016.08.012.

Tocher, D. R., Fonseca-Madrigal, J., Dick, J. R., Ng, W. K., Bell, J. G., & Campbell, P. J. (2004). Effects of water temperature and diets containing palm oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology – B Biochemistry and Molecular Biology*, *137*(1), 49–63. https://doi.org/10.1016/j.cbpc.2003.10.002.

Torno, C., Staats, S., Michl, S. C., De Pascual-Teresa, S., Izquierdo, M., Rimbach, G., & Schulz, C. (2018). Fatty acid composition and fatty acid associated gene-expression in gilthead sea bream (*Sparus aurata*) are affected by low-fish oil diets, dietary resveratrol, and holding temperature. *Marine Drugs*, *16*(10), 379. https://doi.org/10.3390/md16100379.

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O. T., Nilsen, F., … Jackson, D. (2013). Salmon lice – impact on wild salmonids and salmon aquaculture. *Journal of Fish Diseases*, *36*(3), 171–194. https://doi.org/10.1111/jfd.12061.

Treasurer, J. W. (2002). A review of potential pathogens of sea lice and the application of cleaner fish in biological control. *Pest Management Science*, *58*(6), 546–558. https://doi.org/10.1002/ps.509.

Trueman, R. J., Tiku, P. E., Caddick, M. X., & Cossins, A. R. (2000). Thermal thresholds of lipid restructuring and δ9-desaturase expression in the liver of carp (*Cyprinus carpio* L.). *The Journal of Experimental Biology*, *203*(3), 641–650. https://doi.org/10.1242/jeb.203.3.641.

van der Marel, M., Caspari, N., Neuhaus, H., Meyer, W., Enss, M. L., & Steinhagen, D. (2010). Changes in skin mucus of common carp, *Cyprinus carpio* L., after exposure to water with a high bacterial load. *Journal of Fish Diseases*, *33*(5), 431–439. https://doi.org/10.1111/j.1365-2761.2010.01140.x.

Vatsos, I. N., Kotzamanis, Y., Henry, M., Angelidis, P., & Alexis, M. N. (2010). Monitoring stress in fish by applying image analysis to their skin mucous cells. *European Journal of Histochemistry*, *54*(2), 107–111. https://doi.org/10.4081/ejh.2010.e22.

Xie, F., Xiao, P., Chen, D., Xu, L., & Zhang, B. (2012). miRDeepFinder: A miRNA analysis tool for deep sequencing of plant small RNAs. *Plant Molecular Biology*, *80*, 75–84. https://doi.org/10.1007/s11103-012-9885-2.

Ytteborg, E., Hansen, Ø. J., Høst, V., Afanasyev, S., Vieweg, I., Nahrgang, J., & Krasnov, A. (2020). Morphology, transcriptomics and in vitro model of skin from polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*). *Fishes*, *5*(4), 34. https://doi.org/10.3390/fishes5040034.

Ytteborg, E., Lazado, C. C., Noble, C., Hansen, R. I., & Johansen, L. H. (2023). The skin mucosal barrier of lumpfish (*Cyclopterus lumpus L.*) is weakened by exposure to potential aquaculture production related stressors. *Journal of Fish Biology*, *1-15*. https://doi.org/10.1111/jfb.15352.

Yuen, J. W., Dempster, T., Oppedal, F., & Hvas, M. (2019). Physiological performance of ballan wrasse (*Labrus bergylta*) at different temperatures and its implication for cleaner fish usage in salmon aquaculture. *Biological Control*, *135*, 117–123. https://doi.org/10.1016/j.biocontrol.2019.05.007.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kottmann, J. S., Berge, G. M., Kousoulaki, K., Østbye, T.-K. K., Ytteborg, E., Gjerde, B., & Lein, I. (2023). Welfare and performance of ballan wrasse (*Labrus bergylta*) reared at two different temperatures after a preparatory feeding trial with enhanced dietary eicosapentaenoic acid. *Journal of Fish Biology*, 1–18. https://doi.org/10.1111/jfb.15482