Deformities prevalence in farmed ballan wrasse (*Labrus bergylta*) in relation to hatchery origin and life stage

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Abstract

The production of farmed ballan wrasse (*Labrus bergylta*) is developing, with an emphasis on sustainability and quality. However, ballan wrasse hatcheries have anecdotally reported increased prevalence of malformations which may impact on fish welfare and hatchery productivity. The present study therefore aimed to identify and characterise deformities in two of the largest ballan wrasse producers in the UK. A total of 384 farmed fish were sampled at two life stages (post-weaning and pre-deployment) and independent production runs. Additionally, 25 wild caught ballan wrasse were analysed and used as a reference. Each fish was externally examined for malformations including jaw and operculum deformities. The fish were internally examined by x-ray for vertebral deformities and abnormalities of the swim bladder. Mineral analysis of both whole fish and vertebrae were also conducted. The results showed the first information on the ballan wrasse skeleton structure. The total number of vertebrae per fish ranged from 34 to 37, with 37 vertebrae per fish representing 58.2 % of all fish analysed. The vertebral column was divided into two regions, namely R1 and R2. R1 included vertebrae 1 to 17 (post-cranial and pre-haemal vertebrae) while R2 included vertebrae 18 to 34-37 (haemal vertebrae and haemal caudal vertebrae). Results showed a high prevalence of vertebrae malformations (up to 33%), jaw/operculum malformations (up to 13.5 %) and nephrocalcinosis (up to 25 %), with high severity levels in some cases when compared to wild specimens in which malformations were absent. Most malformations were already visible at post-weaning. Wild fish did not show signs of any malformations. Finally, high mineral diets are suggested as a potential route of investigation to reduce the vertebral deformities in ballan wrasse. Increasing the productivity of cleaner fish hatcheries is key to addressing the on-going challenge of sea lice in Atlantic salmon (*Salmo salar*) farming. It is therefore of paramount importance that the causes of the presently identified pathologies are confirmed and mitigation steps introduced.

Keywords: Ballan wrasse; cleaner fish; deformity; nephrocalcinosis; vertebrae.

1. Introduction

With the increase in production of ballan wrasse (*Labrus bergylta*) for biological control of sea lice (Blanco Gonzalez and de Boer, 2017; Brooker *et al*., 2018; Overton *et al.*, 2020), there are increasing anecdotal reports of malformations surfacing. The presence of malformations during production was documented in a recently published study (Fjelldal et al., 2020), however is not unique to this species and occurs in many other commercial species like Atlantic cod (*Gadus morhua*) (Fjelldal *et al*., 2009a; Kjørsvik *et al*., 2009), Atlantic salmon (*Salmo salar*) (Fjelldal *et al*., 2007; Witten *et al*., 2009, 2006; Smedley et al., 2018; Vera et al., 2019), European sea bass (*Dicentrachus labrax*) (Boglione *et al*., 2013a, 2013b) and gilthead seabream (*Sparus aurata*) (Andrades *et al*., 1996; Fernández *et al*., 2008; Boglione and Costa, 2011). In ballan wrasse, from 17 to 53 % of fish sampled from commercial production in Norway showed severe vertebra deformities (≥6 deformed vertebrae) (Fjelldal et al., 2020), which is concerning, but a broader perspective is required across the industry to understand if it is a common challenge faced by the sector or specific to local production practices.

Skeletal deformities in marine fish are typically associated with nutritional deficiencies and/or suboptimal environmental conditions, although a genetic influence cannot be discounted (Divanach *et al*., 1996; Cahu *et al*., 2003b; Zambonino and Cahu, 2010; Boglione and Costa, 2011). Skeletogenesis can be affected during early fish development due to unbalanced levels of nutrients (*e.g.* lipids, minerals, vitamins, amino acids) (Cahu *et al*., 2003a; Lall and Lewis-McCrea, 2007) which can result in long-term and permanent effects on bone health. At larval stages in particular, phospholipids and polyunsaturated fatty acids (PUFA) are of great importance and their dietary deficiencies during this sensitive developmental window were associated with the occurrence of vertebral and jaw deformities in common carp (*Cyprinus carpio*) (Geurden *et al*., 1998) and opercula deformities in

milkfish (*Chanos chanos*) (Gapasin and Duray, 2001). Other nutrients that cause skeletal deformities when deficient are phosphorus in Atlantic salmon (Fjelldal *et al.*, 2012; Smedley *et al*., 2018) and haddock (*Melanogrammus aeglefinus*) (Roy *et al*., 2002), vitamin A in Japanese flounder (*Paralichthys olivaceus*) (Takeuchi *et al*., 1998) and vitamin C in channel catfish (*Ictalurus punctatus*) (Lim and Lovell, 1978). Environmental conditions and rearing practices can also lead to skeletal deformities in marine finfish. For example, inappropriate tank water flow and current have shown to induce lordosis in European sea bass (Divanach *et al*., 1997). Physical parameters such as sub-optimal temperature incubation regimes have shown to considerably affect the skeletogenesis of Atlantic salmon, where higher egg incubation temperatures results in a higher vertebral deformities prevalence (Fraser *et al*., 2015).

Deformities observed in farmed fish are often not seen in the wild populations. In nature, deformed fish tend to have a short life-span, often resulting in the absence or scarcity of deformed adult fish due to poor robustness and/or predation (Branson and Turnbull, 2008). In hatcheries, while sub optimal rearing conditions can increase the apparent abundance of deformities, the protected rearing conditions could also be considered to increase the likelihood of individuals with deformities to survive. Nevertheless, these malformations can result in lower growth performance as deformities such as vertebral malformations or abnormal swimbladders will increase metabolic demand due to compensatory swimming effort at the expense of somatic growth (Boglione *et al*., 2013b). Ultimately, when reduced growth is further confounded with a compromised buoyancy ability or swimming capacity, they are then considered welfare issues that need to be addressed (Huntingford *et al*., 2006). In the case of ballan wrasse, there is a further consideration in that their purpose is for biological control and thus focus on the jaw structure is of particular relevance as malformations can reduce their delousing efficiency (Leclercq *et al*., *unpublished*).

Furthermore, commercial health screenings of farmed wrasse has also reported high abundance of nephrocalcinosis in some populations. This is a chronic inflammatory pathology in which minerals (e.g. calcium, phosphorus) precipitate as hydroxyapatite within the distal renal tubules resulting in the creation of "stones" (Noble *et al.*, 2018, Sandoval, 2019). Nephrocalcinosis has been reported in other species and has previously been categorised as a severe abnormality in fish which can result in growth impairment (Boglione *et al.*, 2001).

Given the anecdotal reports of deformities from UK hatcheries producing ballan wrasse and the recently published data from Norwegian farmed fish (Fjelldal *et al.*, 2020), there is a clear need for a broader screening of both external and internal malformations in the species. The aim of this study was to identify and compare skeletal deformity prevalence, types and severity in ballan wrasse collected from commercial marine hatcheries in the UK to compare to that reported in Norway. A further aim was to document the prevalence of jaw deformities and nephrocalcinosis, which have not been reported yet in the species. In addition, screening was performed at two production life stages to improve our understanding of the aetiology of deformities. The overarching aim of this research is to broaden our awareness of deformity prevalence in farmed ballan wrasse to support the sustainable intensification of its production.

2. Materials and Methods

2.1. Sampling

A total of 384 farmed ballan wrasse were sampled from two commercial hatcheries in Scotland (referred to as Hatchery A "H_A" and B "H_B"), between 2017 and 2018. In each hatchery, three independent batches of two different life stages (post-weaning "PW" and pre-deployment "PD") were sampled (n = 32 per sampling point). With sampling happening over several months in both hatcheries, different production year classes ("YC") were also sampled (YCs 2015, 2016, 2017 and 2018) (Table 1).

Differences between both hatcheries in production husbandry and environmental conditions are presented in Table 2. The mineral content of the diet used during the on-growing, which account for 83 % of the feed given during their hatchery production, are presented in Table 3 The weaning diets, which account for less than 9 % of the feed given during the production, were not presented as there were potentially too many diet manipulations in both hatcheries at this stage.

In both hatcheries, water chemistry was monitored daily for ammonia (<0.3 mg L^{-1}), pH (7.8 - 8.2) and dissolved oxygen (>90 % saturation), remaining within optimal ranges for marine finfish species. Ceramic clay (Kentucky OM #4 Ball Clay, Sheffield Pottery, Sheffield, UK) was used to control tank hygiene and bacteria load in the water at both hatcheries.

An additional 25 wild specimens were caught using baited creels in the Sound of Arisaig (Glenuig, UK) by a professional fisherman in June 2018. All fish were euthanised by overdose of MS-222 (Pharmaq, UK) followed by exsanguination. Then, every fish was weighed (W, 0.1 g), measured (total length, TL, 0.1 cm) and condition factor (K) calculated using Fulton's formula:

$$K = W \times (L^3)^{-1} \times 100$$

136 Photographs of the fish were taken, and external abnormalities were recorded (*i.e.* presence
137 or absence of operculum damage or severe jaw deformity which was defined as being jaws
138 which would not close, Fig. 1). Fish were then flat frozen at -20 ºC until further X-ray
139 analysis.

140 *2.2. Radiography*

141 Radiographs of the frozen fish were taken using a digital mammography x-ray cabinet
142 (UltraFocus, Faxitron Bioptics, LLC, AZ, USA). Fish were placed on a 29 x 23 cm plate and
143 a right lateral view radiological image was taken enabling internal vertebral analysis. Post-
144 weaning fish were exposed to 1 mAs and 25 kV while pre-deployment and wild fish were
145 exposed with 2 mAs and 25 kV. Radiological images of each fish were examined using
146 OsiriX Lite (v.9.0.1, Pixmeo, Geneva, Switzerland) for internal structures including total
147 number of vertebrae, individual vertebrae structure and severity of deformity, swimbladder
148 form (defined as being: absent, normal, under inflated, overinflated or other pathologies
149 (typically multiple chambered)) and presence of presumed nephrocalcinosis (Fig. 2 & 3).

150 All analyses were performed by three people independently with a $^{2}/_{3}$ consensus being
151 required to define each classification. Definitions of the different vertebral regions were
152 established according to Roberts and Ellis (2012). Based on the examination of each
153 individual vertebra (Fig. 2), the vertebral column was divided into two regions, namely R1
154 and R2. R1 included vertebrae 1 to 17, composed of post-cranial and pre-haemal vertebrae,
155 while R2 included vertebrae 18 to 34-37, composed of the haemal vertebrae and haemal
156 caudal vertebrae (Fig. 2). Vertebral deformities were defined and determined based on the
157 methods described in Fjelldal *et al.* (2007, 2009a) and Witten *et al.* (2009).

158 When looking at vertebral deformities, individuals were further reclassified into severity
159 bands in terms of number of deformed vertebrae (dv) affected (*i.e.* 0 dv, 1-3 dv, 4-6 dv and

160 >6 dv). Briefly, the categories were created based on the proportional interpretation to those
161 proposed for Atlantic salmon by Hansen *et al*. (2010) taking into account the different
162 number of vertebrae in the two species (*i.e. circa* 58 and 37 vertebrae in Atlantic salmon and
163 ballan wrasse, respectively). In fish with >6 dv, the deformities were further categorised in
164 terms of consecutive deformed vertebrae, referred to as "blocks" (*i.e.* blocks of 1, 2, 3, 4 or
165 ≥5 dv).

166 *2.3. Mineral analysis*

167 At the post-weaning stage, six individuals per hatchery with 0 dv were selected for mineral
168 analysis. Given there were not enough fish displaying >6 dv per hatchery, the deformed
169 individuals were selected as follows: six individuals displaying 1-3 dv or more for H_A and six
170 individuals displaying 4-6 dv or more for H_B which represents the most severely deformed
171 individuals for each respective hatchery at this life stage. At pre-deployment stage, six
172 individuals with 0 dv and six individuals displaying >6 dv were selected in both hatcheries.
173 For wild fish, only six individuals with 0 dv were selected as there was no fish with >6 dv.

174 All fish were defrosted at room temperature and baked at 110 ºC for 20 mins. The
175 post-weaning fish were then turned into ash in a muffle furnace at 600 ºC overnight, allowing
176 the calculation of their whole-body ash content (see equations below). The larger size of the
177 pre-deployment fish allowed us to dissect out and de-flesh the whole vertebral columns. Each
178 vertebral column was then weighed and baked at 110 ºC overnight. The dry vertebral
179 columns were then cooled in a desiccator, re-weighed and turned into ash in a muffle furnace
180 at 600 ºC overnight. Moisture and ash content of the vertebral column was calculated as
181 follows:

182

183 $$\% \, Moisture = \frac{Sample\ weight\ (g) - Dried\ sample\ weight\ (g)}{Sample\ weight\ (g)} \times 100$$

184

185 $$\% \, Ash = \frac{Ash\ weight\ (g)}{Sample\ weight\ (g, dry\ weight)} \times 100$$

186

187 The ash samples of the vertebral columns, sampled feeds and dissected nephrocalcinosis
188 were individually digested in nitric acid (69 %) in a microwave (MARSXpress, CEM) for 40
189 mins (20 mins ramping to 120 ºC and 20 mins holding that temperature). Digests were
190 transferred into a volumetric flask and made up into x 25 dilutions with distilled water.
191 Samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS,
192 Thermo Scientific Model X Series 2, USA). The mineral content of the post-weaning fish
193 (whole bodies) could not be measured as there was not enough dry material (< 50 mg) to
194 allow replicated analysis (including through pooling).

195 *2.4. Statistical analysis*

196 All data are presented as mean ± standard deviation. Percentage data were subjected to
197 arcsine square-root transformation prior to statistical analyses. Normality and homogeneity of
198 variance of the data were confirmed using Shapiro-Wilk and Levene's tests, respectively.
199 Prevalence and mineral data were analysed by two-way ANOVA (2 locations x 2 stages, $P <$
200 0.05). Condition factor data based on severity (*i.e.* number of deformed vertebrae per
201 individual) was analysed by one-way ANOVA ($P < 0.05$). Wild samples data were provided
202 as a comparator but were not included in the statistical analysis. All data were analysed using
203 SPSS (IBM SPSS Statistics 23, NY, USA) and Microsoft Excel (v16, WA, USA).

3. **Results**

3.1. Vertebral structure of ballan wrasse

The total number of vertebrae per fish ranged from 34 to 37, with 37 vertebrae per fish representing 58.2 % of all fish analysed (i.e. farmed and wild), 36 vertebrae representing 33.8 %, 35 vertebrae representing 7.7 % and 34 vertebrae representing 0.3 %. There was no difference in vertebrae numbers and vertebra position in relation to stock origin.

3.2. Vertebral deformities in ballan wrasse

Among the wild population, 96 % of the fish showed no deformed vertebrae and a maximum prevalence of 0.4 % of total deformed vertebrae was observed (Table 4). Total prevalence of deformed vertebrae was higher in Hatchery B compared to Hatchery A (8.9 ± 1.9 % and 5.0 ± 2.3 %, respectively). However, in both hatcheries, pre-deployment fish had overall more deformed vertebrae than at post-weaning stage (8.1 ± 2.6 % and 5.7 ± 2.8 %, respectively) (Fig. 4 and Table 4). In Hatchery B, fish showed 2.2 and 1.9 times more deformed vertebrae at pre-weaning and pre-deployment stages, respectively, compared to fish in Hatchery A. The majority of the deformed vertebrae were located in two areas, respectively between vertebra 1 and 14 and between vertebra 30 to 37 (Fig. 4). The prevalence of deformed vertebrae in R1 reflected the same trend as the whole vertebral column with both the location and the stage having a significant influence, with deformed vertebrae prevalence being nearly twice as high at pre-deployment and in Hatchery B than at post-weaning and Hatchery A (Table 4). The prevalence in R2 was only significantly affected by the location, being almost double in Hatchery B than in Hatchery A. Regarding the severity, the percentage of fish with no deformities (*i.e.* with 0 dv) was significantly different between locations, with 42 % more non-deformed fish in Hatchery A than in Hatchery B (Table 4). The proportion of severely deformed fish (*i.e.* >6 dv) varied from 3.1 ± 0.0 % (Hatchery A, Post-weaning) to 12.5 ± 9.4

% (Hatchery B, Pre-deployment). Among the fish with >6 dv, only the prevalence of blocks made of 2 dv was significantly affected by the life stage, with 59 % more 2 dv blocks at post-weaning than at pre-deployment (Table 4). There was a statistically significant interaction between hatcheries and life stages on the prevalence of blocks made of ≥5 dv (Table 4). The prevalence of blocks made of ≥5 dv was four times higher at pre-deployment than at post-weaning in both hatcheries with overall prevalence being higher in Hatchery B than Hatchery A (Table 4). The condition factor was not affected by the severity of vertebral deformity (Table 5).

3.3. Minerals

The total mineral content of the post-weaning whole fish bodies and the pre-deployment vertebral columns was affected by the source hatchery but not by the severity level (Fig. 5 and 6). The total mineral content of the whole body of post-weaning fish was significantly higher (+9.8 %, $P < 0.05$) in Hatchery B than in Hatchery A fish (Fig. 5). The total mineral content of the vertebral column of pre-deployment fish was significantly higher in the wild fish (+16.1 %) and Hatchery A (+9.7 %) than Hatchery B (Fig. 6). In addition, an extensive range of macro- and microminerals were analysed but the majority did not show any relevant differences, with only magnesium, phosphorus, calcium and zinc, due to their relevance to bone development, presented (Table 6). The levels of magnesium, phosphorus and calcium of the vertebrae of the pre-deployment fish were affected by the location but not by the severity (Table 6). Magnesium levels in the vertebrae of the fish from Hatchery B were 66.4 and 34.0 % higher than in the wild and Hatchery A fish, respectively. Phosphorus levels were 31.2 and 36.9 % higher in the vertebrae of the fish from the wild and Hatchery A, respectively, compared to that of Hatchery B fish. Calcium levels were 38.1 % and 42.4 % higher in the vertebrae of the fish from the wild and Hatchery A, respectively, compared to that of Hatchery B fish. The ratio Ca:P remained constant across groups, ranging between 1.8 ± 0.1

253 (H_B – 6 dv) and 1.9 ± 0.2 (H_A – 0 dv) in absolute values which equates to 1.4 ± 0.1 (H_B – 6
254 dv) and 1.5 ± 0.1 (H_A – 0 dv) in molar ratio . The zinc levels were also comparable between
255 groups, ranging between 109.4 ± 23.8 µg g^{-1} (H_B – 0 dv) to 128.9 ± 29.7 µg g^{-1} (H_A – 6 dv).

256 *3.4. Characterisation of external and internal deformities other than vertebral*
257 *deformities*

258 There was a substantial variability in the length, weight and condition factor of the sampled
259 fish (Table 1). The condition factor of pre-deployment fish was consistently higher in the
260 cultured fish (1.8 ± 0.1) than in the wild fish (1.5 ± 0.1). Among external deformities, the
261 prevalence of jaw malformation (Fig. 1A and B) was different between the locations and the
262 stage of development, while operculum malformation (Fig. 1C and D) was only different
263 between the locations (Table 7). Overall, the prevalence of jaw deformity in Hatchery B (22.9
264 ± 12.3 %) was over 4 times higher than in Hatchery A (5.2 ± 4.3 %) and accounted for up to
265 one third of the pre-deployment fish in Hatchery B. The prevalence of jaw deformity was 2.6
266 times higher at pre-deployment (20.3 ± 15.1 %) than at post-weaning (7.8 ± 5.8 %). Finally,
267 the prevalence of deformed operculum was more than 8 times higher in Hatchery B than in
268 Hatchery A, accounting for up to 13.5 ± 9.5 % of the pre-deployment fish in Hatchery B
269 (Table 7). There were no observed deformities of operculum or jaws in the wild fish
270 examined.

271 With respect to internal deformities other than vertebral deformities, the percentage of
272 normally developed swimbladders ranged from 51.0 ± 46.4 % at post-weaning in Hatchery B
273 to 95.8 ± 7.2 % at pre-deployment in Hatchery A. Only pathologies such as “multichambered
274 swimbladder” were specifically associated to post-weaning stage (no interaction).
275 Nephrocalcinosis was observed in both hatcheries and stages, ranging from 10.4 ± 11.0 %
276 (Hatchery B at pre-deployment) to 28.1 ± 9.4 % (Hatchery A at pre-deployment). The
277 analysis of a sampled nephrocalcinosis showed the following minerals: calcium (55.49 %),

278 phosphorus (42.35 %), magnesium (1.57 %), sodium (0.37 %), potassium (0.22 %) and trace
279 minerals (<0.0001 %). There was no evidence of swimbladder deformities or
280 nephrocalcinosis in all wild fish examined (Table 7). There was no identifiable relationship
281 between the severity of vertebral deformity and the prevalence of the external and internal
282 deformities identified, furthermore there was no assoacition in presence of jaw and opercular
283 deformities.

4. **Discussion**

As the demand for farmed ballan wrasse is increasing, concerns for quality of the deployed individuals is growing. Intensification of production in any marine finfish typically comes with an increase in the number of malformations and abnormalities that can result in reduced growth (Boglione *et al*., 2013a; Boglione and Costa, 2011) and ultimately compromise fish welfare (Huntingford *et al*., 2006). The first published scientific reports confirmed this concern in farmed ballan wrasse in Norway (Fjelldal *et al.* 2020) however, there is no scientific evidence published on vertebral deformity prevalence, typology and severity as well as qualification of jaw/opercula, swimbladder and nephrocalcinosis deformity prevalence in UK hatcheries. To ensure the hatcheries' long-term productivity as well as the efficiency and welfare of the deployed animals, an evaluation of the current production in Scotland was required to identify the main deformities and compare with the results from similar studies on farmed stocks in Norway, so their aetiology could be considered, and mitigation strategies proposed.

Skeletal deformities are among the most common malformations found in farmed finfish. They have been extensively investigated in species with high commercial value such as Atlantic salmon (Fjelldal *et al*., 2012, 2007; Witten *et al*., 2009; Smedley et al., 2018; Vera et al., 2019), European sea bass (Chatain, 1994; Divanach *et al*., 1997; Boglione *et al*., 2013a, 2013b) and Atlantic cod (Fjelldal *et al*., 2009a). In order to do the same with ballan wrasse and be able to compare it to that of the above-mentioned species, defining the vertebral anatomy of the species was required. The ballan wrasse sampled during the study, either wild or farmed, had between 34 to 37 vertebrae in agreement with Fjelldal et al. (2020). In comparison, Atlantic salmon has 58 vertebrae (Witten *et al*., 2009), European sea bass 25 (Kranenbarg *et al*., 2005) and Atlantic cod 52 (Fjelldal *et al*., 2013), showing a great diversity among marine species. The radiological study showed that farmed ballan wrasse were subject

309 to vertebral deformities, with a higher prevalence among the pre-deployment populations *i.e.*
310 the prevalence increased through hatchery production. The majority of the deformed
311 vertebrae were located in two distinct areas, within R1 corresponding to the abdominal
312 section (vertebrae 1 to 14) and R2 corresponding to the tail region (vertebra 30 to 37). While
313 trunk deformities in the current study agree with recently published data (between vertebra 4
314 and 10), contrasting results are reported for the tail region (between vertebra 19-26, mainly
315 compression type) (Fjelldal et al., 2020). With respect to R1 deformities, as this region was in
316 close alignment to the swimbladder, 91 % of the pre-deployment fish had normally inflated
317 swimbladders whereas the prevalence of normal swimbladders was considerably lower at
318 post-weaning stage (61 %), which may be due to prior mortality of deformed fish. In ballan
319 wrasse, the inflation of the swimbladder occurs at around 13 days post hatch (D'Arcy *et al*.,
320 2012). Hatcheries have reported fish with over-inflated swimbladders at early developmental
321 stages that appear to recover later on (Brooker *et al*., 2018). However, it is possible that
322 during those events, the over-inflated swimbladder compresses the vertebral column,
323 consequently deforming the vertebrae with long-term effects, as observed in Atlantic salmon
324 (Grotmol *et al*., 2005; Fjelldal *et al*., 2009b). While several biotic and abiotic factors are
325 known to impact swimbladder inflation (Woolley and Qin, 2010), contaminants e.g. oils in
326 tank surface water is one of the main drivers which can be addressed through the use of
327 surface skimmers (Chatain and Ounais-Guschemann, 1990). As is routine for most marine
328 hatcheries, both study sites used surface skimmers, yet the study showed that some fish still
329 had problems developing normal swimbladders. This suggests that the efficacy of surface
330 skimmer deployment along with other optimum conditions for swimbladder development and
331 inflation in ballan wrasse require further investigation to identify other possible causative
332 drivers of swimbladder malformation. The second area with high deformity prevalence was
333 located in the tail region, between vertebrae 32 and 37 as opposed to 19-26 reported in

farmed ballan wrasse in Norway (Fjelldal et al., 2020). Sub-optimal water dynamics (*e.g.* current intensity, flow direction, etc.) can provoke higher swimming activity that may result in excessive pressure on the vertebrae of that region, leading to the development of deformities in European sea bass (Chatain, 1994). With no information regarding the swimming dynamics for the species, specific cause and effect cannot be concluded for ballan wrasse. However, given that ballan wrasse are reef fish that do not face the same current flow and strength as salmon for example, it is possible that tank flow dynamics require closer attention. It could be possible that testing turbulent flows instead of unidirectional flows in the tanks may be more suited to the species and help mitigate the prevalence of deformities in this vertebral region. Importantly, in contrast to farmed fish, wild ballan wrasse did not have many deformities, either externally nor internally (except for one fish with 4 dv). Fjelldal et al. (2020) reported a higher prevalence of vertebra deformity in wild wrasse captured in Norway (11% with ≥1 and 1% with ≥6 deformed vertebrae with 83 % of fish showing calluses and 14% fractures in haemal/neural vertebral columns and/or ribs). It is important to note that both Fjelldal's study and the hereby study used the same deformity key (Witten *et al.*, 2009) thus it is fair to presume that the variation observed between the Norwegian and Scottish populations are not due to differences in the scoring methodology. Ribs, and neural and haemal arches of the vertebral columns were not assessed in the current study therefore no comparison can be made on these points. It is fair to presume that in the wild, physical abnormalities will hamper the individual's capacity to swim properly, feed or escape a predator, thus reducing its likelihood to survive. Correspondingly, fish with deformities have a greater chance to survive in the hatcheries with controlled environmental conditions, available food and absence of predation.

Vertebral deformities in marine finfish, and farmed Atlantic salmon in particular, have been extensively investigated and reviewed (Divanach *et al.*, 1996; Witten *et al.*, 2009,

2006; Boglione *et al*., 2013b). For Atlantic salmon, Hansen (2010) has set a critical threshold to 10 dv per individual, which was shown to significantly reduce growth rate and impact on welfare of the fish. Since ballan wrasse have less vertebrae than Atlantic salmon, we hereby applied a proportional adjustment that resulted in a proposed severity threshold of >6 dv for the species in agreement Fjelldal et al. (2020). While we used this threshold as a proxy for ballan wrasse in the current study, further work is required to validate this threshold by characterising precisely the structure of a "normal" vertebral column in the species and defining the subsequent impact of vertebral deformities on growth and welfare of the species. At pre-deployment stage, the average proportion of fish with severe (>6 dv) vertebral deformity was notable in both hatcheries (i.e. >10 %). Interestingly, it appears that the Norwegian production of farmed ballan wrasse has a greater prevalence, with 17 to 53 % of the farmed fish presenting severe vertebra deformities (*i.e.* ≥6 dv) (Fjelldal *et al.*, 2020). While many causative factors are potentially associated with this (*e.g.* feeding regime in larval rearing and weaning), one notable difference is the thermal rearing protocols utilised. In Norway, early rearing protocols typically raise temperatures to 16 °C (Høyland 2015) whereas in UK hatcheries (including the present study) temperatures during live feed rearing remain at around 12 °C. Given the impact of suboptimal high temperatures during early life stages on vertebral deformities in other finfish species (Fraser *et al*., 2015; Clarkson et al., in press), the authors propose defining the impact of rearing temperature on the prevalence of vertebral deformity is a priority research area as gains in growth performance may be to the detriment of animal welfare. Further analysis revealed a higher prevalence of blocks of ≥5 dv in pre-deployment fish whereas blocks of 2 dv were more prevalent at post-weaning. Multiple successive deformed vertebra may have a more detrimental effect on the fish growth and welfare compared to that of multiple single deformed vertebrae spread across the

383 vertebral column (Witten *et al*., 2006, 2009). Further investigation on the specific aetiology
384 of these >5 dv block deformities is required to then reduce their prevalence.

385 The mineral analysis at post-weaning (*i.e.* whole body) and pre-deployment (*i.e.*
386 vertebral column) showed that total mineral content was different between hatcheries but did
387 not associate with observed severity. While it is acknowledged that vertebral columns were
388 not de-fatted prior to analysis it is presumed given that ballan wrasse are "lean" species the
389 bone fat content would be *circa* 3% or less and thus have minimal influence over the
390 observed results (Toppe et al. 2007). Interestingly, the mineral levels in fish from Hatchery A
391 were comparable to that of wild samples, in which no deformities were found. Among
392 macrominerals, phosphorus, magnesium and calcium are often seen as the most critical as
393 they directly impact on bone mineralisation in fish (Boglione *et al*., 2013a, 2013b;
394 Baeverfjord *et al*., 2018; Smedley *et al*., 2018). Phosphorus and calcium are structural
395 components of hard tissues (*e.g.* bone, scales, teeth) while magnesium plays an important
396 role in skeletal tissue metabolism, osmoregulation and neuromuscular transmission, among
397 other processes (Roy *et al*., 2002; Lall & Lewis-McCrea 2007; Lewis-McCrea & Lall 2010;
398 NRC 2011). Importantly, P and Ca levels in the vertebral column were inversely related to
399 the dietary Mg level, suggesting that Mg may reduce Ca absorption (Liang *et al*., 2012). This
400 could explain the lower levels of Ca in the vertebral column of fish from Hatchery B while
401 Mg levels were higher than in Hatchery A. Fish sampled in the present study were fed using
402 different diets and feeding regimes between hatcheries although Hatchery A was using the
403 Otohime feeds while Hatchery B used the Symbio feeds. Phosphorus and calcium levels were
404 higher in Hatchery A with the whole-body Ca levels reflecting the higher dietary Ca found in
405 the Otohime diet. However, magnesium levels were higher in fish from Hatchery B and not
406 clearly associated with dietary differences. The mineral requirements for finfish are typically
407 about 3 to 9 g kg^{-1} for phosphorus, 3 g kg^{-1} for calcium and 4 to 6 g kg^{-1} for magnesium, yet

these remain highly species specific (Lall, 2003; NRC, 2011). Both hatcheries diets were therefore several-fold higher than the P and Ca requirements but below the requirements for Mg. High mineral diets have shown to reduce the risk of developing vertebral deformities in under-yearling Atlantic salmon (Fjelldal *et al*., 2009b; Vera et al., 2019) thus one factor that could explain the lower prevalence of vertebral deformities in Hatchery A is that they were fed a diet richer in mineral (*i.e.* Otohime). Further investigation is required to determine the optimal dietary mineral requirements, with particular attention to magnesium, as well as the sensitive developmental windows for the species to confirm this hypothesis, as proposed by Hamre *et al*. (2013).

Regarding the external appearance of the fish, the condition factor was not affected by the severity of the vertebral deformities thus K would not be a good proxy for vertebral deformities. However, K was consistently higher in the farmed individuals compared to wild fish, which is in line with other studies on the species (Leclercq *et al*., 2014a and b, Cavrois-Rogacki *et al*., 2019). The controlled conditions in the hatcheries (*e.g.* constant water flow) as well as the non-limited feed are often seen as the main drivers behind increased condition in farmed fish. The occurrence of jaw deformities was higher at pre-deployment and in Hatchery B, representing up to 1/3 of the fish in some batches. A functional jaw is crucial for the wrasse delousing capabilities. Currently, fish with severely deformed jaws are culled, constituting a notable loss for the hatcheries, particularly if the animals are not identified until the pre-deployment stage. Similarly, deformed operculum accounted for more than 20 % of some batches, with a higher prevalence in Hatchery B. As the operculum acts as a physical barrier to the gill, deformed or missing operculum might favour gill pathologies such as amoebic gill disease. It has been suggested that jaw and operculum malformations develop mainly during the early larval stages as, in other fish species, it has been associated with sub-optimal thermal regimes during egg incubation (Fraser *et al*., 2015, Imsland *et al*., 2019),

433 sub-optimal environmental conditions (Castro-González *et al*., 2008), dietary deficiencies
434 (Lall and Lewis-McCrea, 2007; Zambonino and Cahu, 2010), live feed enrichment quality
435 (Cahu *et al*., 2003a) and physical environment (*i.e.* tank colour) (Cobcroft and Battaglene,
436 2009; Cobcroft *et al*., 2012). Jaw and operculum deformities can also have a genetic origin
437 (Afonso *et al*., 2000), however, it is unlikely in the current study given that fish originated
438 from different broodstock populations with different genetic history. Present data showed
439 both jaw and operculum deformities were less prevalent at the pre-deployment stage in
440 Hatchery A (7.3 and 2.1 %, respectively) than B (33.3 and 13.5 %, respectively). However,
441 with respect to the physical rearing environments, there are no clear common factors as
442 Hatchery A was operating in recirculation, with temperature control, whereas Hatchery B
443 was operating in flow-through with limited control over temperature.

444 Equally, hatcheries operated with different coloured tanks (light blue vs. dark green),
445 with unknown tank wall brightness characteristics, suggesting walling behaviour may have
446 been a contributing factor to the observed difference in jaw deformity prevalence (Cobcroft et
447 *al.*, 2012; Sawada et *al.*, 2020). One potential factor that has not been fully explored yet
448 would be stocking density that is known to impact on fin condition in fish (Noble *et al.*,
449 2012) and can be very variable in ballan wrasse hatcheries depending on stock survival rates.
450 However, no reliable data on stocking density was available during the production cycles,
451 other than maximum permitted limits. Given the diversity of potential factors shown to
452 influence jaw and operculum deformity, if levels of prevalence are considered commercially
453 and ethically concerning, dedicated research is required to prioritise the causes of these
454 deformities in ballan wrasse, working towards the implementation of mitigation strategies.

455 Finally, almost every batch of fish surveyed by radiography displayed presumed
456 nephrocalcinosis, with more than 25 % of the fish affected in some cases but without
457 apparent differences between hatcheries. This has not been documented previously in the

458 species. Although mortality has not been associated to the presence of nephrocalcinosis, it is
459 suspected that it may affect the fish's osmotic balance. Studies have shown correlations
460 between nephrocalcinosis and growth impairment, poor feed efficiency and other pathologies
461 in spotted wolfish (*Anarhichas minor*) (Foss *et al*., 2003), Atlantic salmon (Gil Martens *et al*.,
462 2006) and rainbow trout (*Onchorynchus mykiss*) (Harrison and Richards, 1979), though its
463 impacts on ballan wrasse physiology remain to be determined. Sub-optimal water chemistry
464 and unbalanced feeds, especially in terms of mineral content, are considered to be potential
465 sources of nephrocalcinosis, even if at this stage it remains speculative given the lack of
466 standardised protocols between hatcheries which prevents comparison. Although its aetiology
467 remains unknown in ballan wrasse, causative agents identified in rainbow trout, that would be
468 worthy of investigation include: high levels of carbon dioxide in the water (*i.e.* >20 mg l^{-1}),
469 magnesium deficiency and low dietary minerals (Roberts and Ellis, 2012).

470 To conclude, this study broadens the evidence base on the prevalence and severity of
471 deformities in farmed ballan wrasse including vertebrae malformations, jaw/operculum
472 malformation and nephrocalcinosis. This work demonstrates that challenges of fish
473 deformities during production is an international issue. However, through comparative
474 analysis of production methods it should be possible to prioritise interventions for
475 remediation e.g. rearing temperatures, diets. Importantly the current work demonstrates that
476 the majority of deformities reported both in the UK and Norway is present in fish at the post
477 weaning stage, requiring future work to be directed to the early larval rearing and live feed
478 protocols as likely causative factors. Ultimately, while increasing the productivity of cleaner
479 fish hatcheries is key to addressing the ongoing challenge of sea lice in Atlantic salmon
480 farming, this aspiration to increase production should not be to the detriment of farmed
481 animal welfare, production of good quality fish for the customer and for the economic
482 profitability of the hatcheries.

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691 **Tables**

692 **Table 1.** Morphometric data and condition factor (K) in wild and cultured ballan wrasse. Data are presented as mean ± SD within a

693 batch (n = 32 and 25 for farmed and wild, respectively).

694

Location	Stage	Batch	YC	Age (months)	n	Weight (g)	Length (cm)	K
Wild	Pre-deployment				25	83.5 ± 23.7	17.7 ± 1.8	1.5 ± 0.1
Hatchery A	Post-weaning	1	2018	4	32	0.3 ± 0.1	2.5 ± 0.3	1.5 ± 0.2
		2	2018	6	32	0.8 ± 0.2	3.8 ± 0.3	1.5 ± 0.2
		3	2017	6	32	0.7 ± 0.2	3.3 ± 0.3	2.0 ± 0.2
					Mean	0.6 ± 0.3	3.2 ± 0.6	1.7 ± 0.3
	Pre-deployment	1	2015	20	32	60.7 ± 18.6	14.9 ± 1.3	1.8 ± 0.2
		2	2015	20	32	73.4 ± 25.5	15.8 ± 1.5	1.8 ± 0.2
		3	2015	20	32	51.7 ± 12.7	14.3 ± 1.1	1.7 ± 0.1
					Mean	61.9 ± 10.9	15.0 ± 0.8	1.8 ± 0.0
Hatchery B	Post-weaning	1	2017	7	32	0.9 ± 0.2	4.1 ± 0.5	1.3 ± 0.2
		2	2017	5	32	0.7 ± 0.2	3.9 ± 0.4	1.2 ± 0.2
		3	2017	6	32	0.6 ± 0.3	3.6 ± 0.6	1.2 ± 0.2
					Mean	0.7 ± 0.1	3.9 ± 0.2	1.2 ± 0.0
	Pre-deployment	1	2016	22	32	49.6 ± 12.7	13.6 ± 1.1	1.9 ± 0.2
		2	2016	22	32	48.8 ± 9.7	13.5 ± 0.9	1.9 ± 0.2
		3	2016	22	32	24.9 ± 7.4	11.0 ± 0.9	1.8 ± 0.2
					Mean	41.1 ± 14.1	12.7 ± 1.5	1.9 ± 0.1

SD: standard deviation; YC: year class.

695 **Table 2.** Hatchery specific rearing protocols for ballan wrasse.
696

Hatchery	A	B
Broodstock		
Origin	Wild caught, SW England & West Scotland	Wild caught, West Scotland
Diet	BioMar Symbio Broodstock	Fresh sausage made of commercial aquafeed fish meal and marine oil (EPA+DHA)
Egg incubation		
Temperature	12 °C up to hatching	12 °C up to hatching
Larvae		
System	RAS	Flow through
Temperature	12 °C	10-12 °C
Stocking densities*	80-100 larvae l^{-1}	80-100 larvae l^{-1}
Tank set-up	7 m^3, flat bottom, light blue coloured, surface skimmer.	9 m^3, semi-conical, green coloured, surface skimmer.
Flow rate	15 (0 DPH) to 116 l min^{-1} (weaning)	25 (0 DPH) to 100 l min^{-1} (weaning)
Volume exchange	13 % h^{-1} (0 DPH) to 99 % h^{-1} (weaning).	17 % h^{-1} (0 DPH) to 67 % h^{-1} (weaning).
Live feeds	First feeding to 25 DPH on enriched rotifers (Ori-Green, Skretting, Norway) at approx. 10 rotifers ml^{-1} followed by enriched *Artemia* (Ori-Green, Skretting, Norway) from 25 to 60 DPH at 2-3 nauplii ml^{-1}.	First feeding to 25 DPH on enriched 10 rotifers ml^{-1} rotifers (Ori-Green, Skretting) followed by enriched *Artemia* (Larviva Multigain, BioMar) from 25 to 80 DPH at 2-3 nauplii ml^{-1}.
Weaning	Nofima (Norway) formulated weaning diet.	50:50 mix of Sparos formulated weaning diet (Sparos, Portugal) and Otohime weaning diet (Marubeni Nissin Feed Co., Japan)
Use of clay	Yes	Yes
Juvenile		
System	RAS	Flow through
Temperature	12-15 °C	10-12°C
Light	Continuous	Continuous
Max. stocking density*	18 kg m^3	20 kg m^3
Tank size	7 m^3 (pre on-growing) then 50 m^3, flat bottom, light green coloured	9 m^3, flat bottom, Green coloured.
Flow rate	116 l min^{-1} (pre on-growing) then 833 l min^{-1}	150 l min^{-1}
Volume exchange	100 % h^{-1}	100 % h^{-1}
Feed	Otohime marine diet range (Marubeni Nissin Feed Co., Japan).	BioMar Symbio feed (BioMar, Denmark)

697 *Maximum stocking densities in each hatchery.
698

699 **Table 3.** Mineral composition of the on-growing diets used in the two hatcheries.
700

	Otohime	BioMar Symbio
Macro minerals (g/kg)		
Na	8.6	5.8
Mg	3.1	2.4
P	21.3	13.6
K	10.2	6.0
Ca	23.0	16.8
Micro minerals (mg/kg)		
V	1.0	2.3
Cr	0.9	0.6
Mn	52.7	30.6
Fe	749.2	194.6
Co	0.5	0.1
Ni	0.7	0.7
Cu	36.4	23.8
Zn	226.3	195.8
Se	2.8	2.9

701
702

Table 4. Prevalence of deformed vertebrae, severity and deformed vertebrae blocks in wild and cultured ballan wrasse. Data are presented as mean ± SD (n = 3) and were analysed by two-way ANOVA (2 locations "L" x 2 stages "S"; **P* < 0.05). Wild samples data are provided as a comparator but were not included in statistical analysis.

Location	Wild	Hatchery A		Hatchery B		Significance		
Stage	Pre-deployment	Post-weaning	Pre-deployment	Post-weaning	Pre-deployment	L	S	L x S
Prevalence (% of dv)								
Total	0.4	3.6 ± 0.5	6.4 ± 2.6	7.9 ± 2.2	9.8 ± 1.3	*	*	ns
R1	0.2	1.2 ± 0.4	4.1 ± 2.4	2.7 ± 0.8	6.6 ± 1.1	*	*	ns
R2	0.2	2.4 ± 0.6	2.3 ± 0.3	5.2 ± 1.7	3.2 ± 0.2	*	ns	ns
Severity (% of fish)								
0 dv	96.0	43.8 ± 13.6	36.5 ± 13.0	24.0 ± 12.6	10.4 ± 1.8	*	ns	ns
1-3 dv	0.0	47.9 ± 14.4	39.6 ± 1.8	40.6 ± 11.3	45.8 ± 3.6	ns	ns	ns
4-6 dv	4.0	5.2 ± 1.8	13.5 ± 4.8	24.0 ± 12.6	31.3 ± 10.8	ns	ns	ns
>6 dv	0.0	3.1 ± 0.0	10.4 ± 10	11.5 ± 1.8	12.5 ± 9.4	ns	ns	ns
Blocks (% of blocks)[1]								
1 dv	100.0	25.0 ± 0.0	25.0 ± 0.0	26.7 ± 2.9	35.0 ± 13.2	ns	ns	ns
2 dv	0.0	66.7 ± 14.4	50.0 ± 43.3	35.0 ± 37.7	10.0 ± 17.3	ns	*	ns
3 dv	0.0	0.0 ± 0.0	8.3 ± 14.4	13.3 ± 12.6	13.3 ± 12.6	ns	ns	ns
4 dv	0.0	8.3 ± 14.4	8.3 ± 14.4	10.0 ± 13.2	10.0 ± 13.2	ns	ns	ns
≥5 dv	0.0	0.0 ± 0.0	8.3 ± 14.4	15.0 ± 13.2	31.7 ± 16.1	ns	*	*

[1]Only among fish with >6 dv.

dv: deformed vertebrae; L: location; ns: not significant; S: stage.

706 **Table 5.** Condition factor (K) based on the severity level in wild and cultured ballan wrasse.
707 Data are presented as mean ± SD and were analysed by one-way ANOVA with severity as
708 factor ($P < 0.05$), *n.b.* no significant differences identified. Wild samples data were provided
709 as a comparator but were not included in statistical analysis. There were no wild fish with
710 neither 1-3 dv or >6 dv thus the lack of condition factor.

Location	Wild	Hatchery A		Hatchery B	
Stage	Pre-deployment	Post-weaning	Pre-deployment	Post-weaning	Pre-deployment
Severity (dv)					
0	1.5 ± 0.1	1.7 ± 0.3	1.7 ± 0.1	1.2 ± 0.3	1.8 ± 0.2
1-3	na	1.6 ± 0.3	1.8 ± 0.2	1.2 ± 0.2	1.9 ± 0.2
4-6	1.43	1.7 ± 0.4	1.7 ± 0.3	1.3 ± 0.2	1.9 ± 0.2
>6	na	1.7 ± 0.0	1.9 ± 0.1	1.3 ± 0.2	2.0 ± 0.2

dv: deformed vertebrae; na: not applicable.

711 **Table 6.** Mineral content of the vertebral column in ballan wrasse with or without vertebral
712 deformities from different locations. Data are presented as mean ± SD (n = 6) and were
713 analysed by two-way ANOVA (2 locations × 2 severities; $P < 0.05$). Superscripts denote
714 significant differences within each column between values. Wild samples data were provided
715 as a comparator but were not included in the statistical analysis.

716

Location	Severity	Mg (mg g^{-1})	P (mg g^{-1})	Ca (mg g^{-1})	Ca:P	Zn (µg g^{-1})
Wild	0 dv	3.5 ± 0.1	242.7 ± 14.5	468.7 ± 22.6	1.9 ± 0.0	111.9 ± 18.4
Hatchery A	0 dv	4.8 ± 1.3^{a}	257.1 ± 61.0^{b}	497.2 ± 128.7^{b}	1.9 ± 0.2	117.1 ± 22.0
	6 dv	3.9 ± 1.7^{a}	249.4 ± 67.4^{b}	469.6 ± 129.1^{b}	1.9 ± 0.0	128.9 ± 29.7
Hatchery B	0 dv	6.0 ± 1.0^{b}	179.3 ± 4.4^{a}	330.9 ± 5.7^{a}	1.8 ± 0.0	109.4 ± 23.8
	6 dv	5.7 ± 0.3^{b}	190.7 ± 7.3^{a}	348.0 ± 9.9^{a}	1.8 ± 0.1	109.5 ± 6.7

Ca:P: Calcium:Phopshorus ratio; dv: deformed vertebrae.

717 **Table 7.** Prevalence (%) of external and internal deformities in wild and cultured ballan wrasse. Data are presented as mean ±
718 standard deviation (n = 3) and were analysed by two-way ANOVA (2 locations "L" x 2 stages "S"; *P < 0.05, **P < 0.001).

Location	Wild	Hatchery A		Hatchery B		Significance		
Stage	Pre-deployment	Post-weaning	Pre-deployment	Post-weaning	Pre-deployment	L	S	L x S
External deformities								
Jaw (%)	0.0	3.1 ± 3.1	7.3 ± 4.8	12.5 ± 3.1	33.3 ± 6.5	**	*	ns
Operculum (%)	0.0	0.0 ± 0.0	2.1 ± 1.8	4.2 ± 4.8	13.5 ± 9.5	*	ns	ns
Swim bladder								
Absence (%)	0.0	1.0 ± 1.8	0.0 ± 0.0	1.0 ± 1.8	0.0 ± 0.0	ns	ns	ns
Normal (%)	100.0	71.9 ± 22.5	95.8 ± 7.2	51.0 ± 46.4	85.4 ± 6.5	ns	ns	ns
Under inflated (%)	0.0	4.2 ± 7.2	4.2 ± 7.2	2.1 ± 1.8	14.6 ± 6.5	ns	ns	ns
Over inflated (%)	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	ns	ns	ns
Other pathologies (%)	0.0	22.9 ± 17.2	0.0 ± 0.0	12.5 ± 16.5	0.0 ± 0.0	ns	*	ns
Nephrocalcinosis (%)	0.0	17.7 ± 23.0	28.1 ± 9.4	22.9 ± 17.2	10.4 ± 11.0	ns	ns	ns

dv: deformed vertebrae; L: location; ns: not significant; S: severity.

Figures

Figure 1. Illustration of normal jaw (A), severely deformed jaw (B), normal operculum (C) and deformed operculum (D) in ballan wrasse.

Figure 2. Normally developed ballan wrasse at pre-deployment stage (A) and its corresponding x-ray (B). ballan wrasse at pre-deployment stage (C) showing vertebral deformities and presence of nephrocalcinosis (D). Alignment of the vertebrae from the normally developed ballan wrasse mentioned above, presenting the anterior face of each vertebrae (E).

Figure 3. Typical examples of x-radiographs of farmed ballan wrasse with a normal swim bladder (A), a missing swim bladder (B), an under inflated swim bladder (C), an over inflated swim bladder (D) and a multi-chambered swim bladder (E).

Figure 4. Prevalence of deformed vertebra along the vertebral column in A) Hatchery A and B) Hatchery B, with the wild fish data presented in both figures as a comparison. The vertebral column has been divided into two regions (R1 and R2) as defined in the Materials and Methods. Each series expresses the relative deformities prevalence of each vertebrae among a batch (n = 32). Three production batches (1 to 3) were analysed for each life stage (post-weaning and pre-deployment).

Figure 5. Total mineral content (% whole body dry weight) of post-weaning ballan wrasse with either 0 dv (Hatchery A and B), 1-3 or more dv (Hatchery A) or 4-6 or more dv (Hatchery B) (dv: deformed vertebrae). Data are presented as mean ± SD (n = 6). Data were analysed by two-way ANOVA (2 locations × 2 severities; $P < .05$). Superscripts denote significant differences between the location and the severity.

Figure 6. Total mineral content (% bone dry weight) of the two vertebral regions of pre-deployment ballan wrasse with either 0 dv or >6 dv. Data are presented as mean ± SD (n = 6).

743 Data were analysed by two-way ANOVA (2 locations × 2 severities; $P < .05$). Superscripts
744 denote significant differences between the location and the severity. Wild samples data were
745 provided as a comparator but were not included in statistical analysis.

Figure 1.

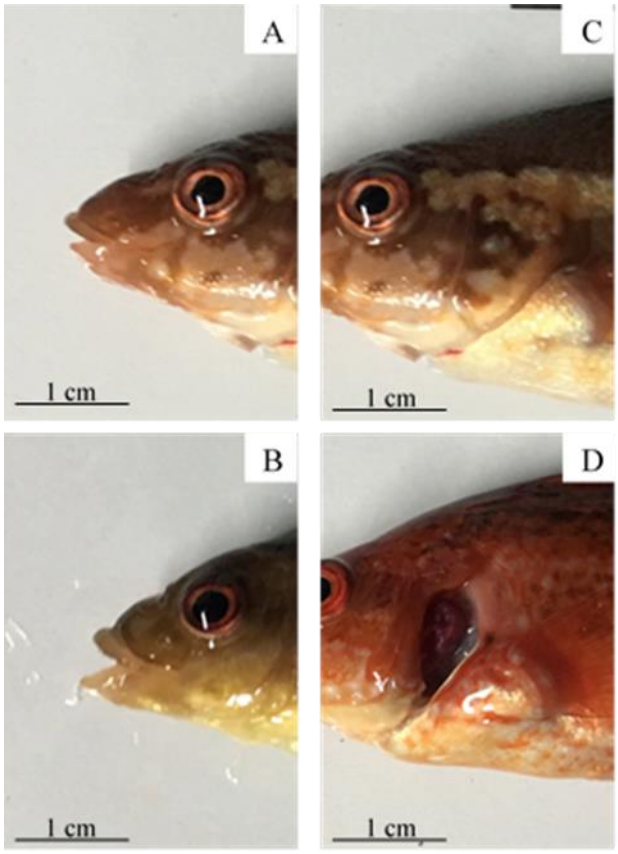


Figure 2.

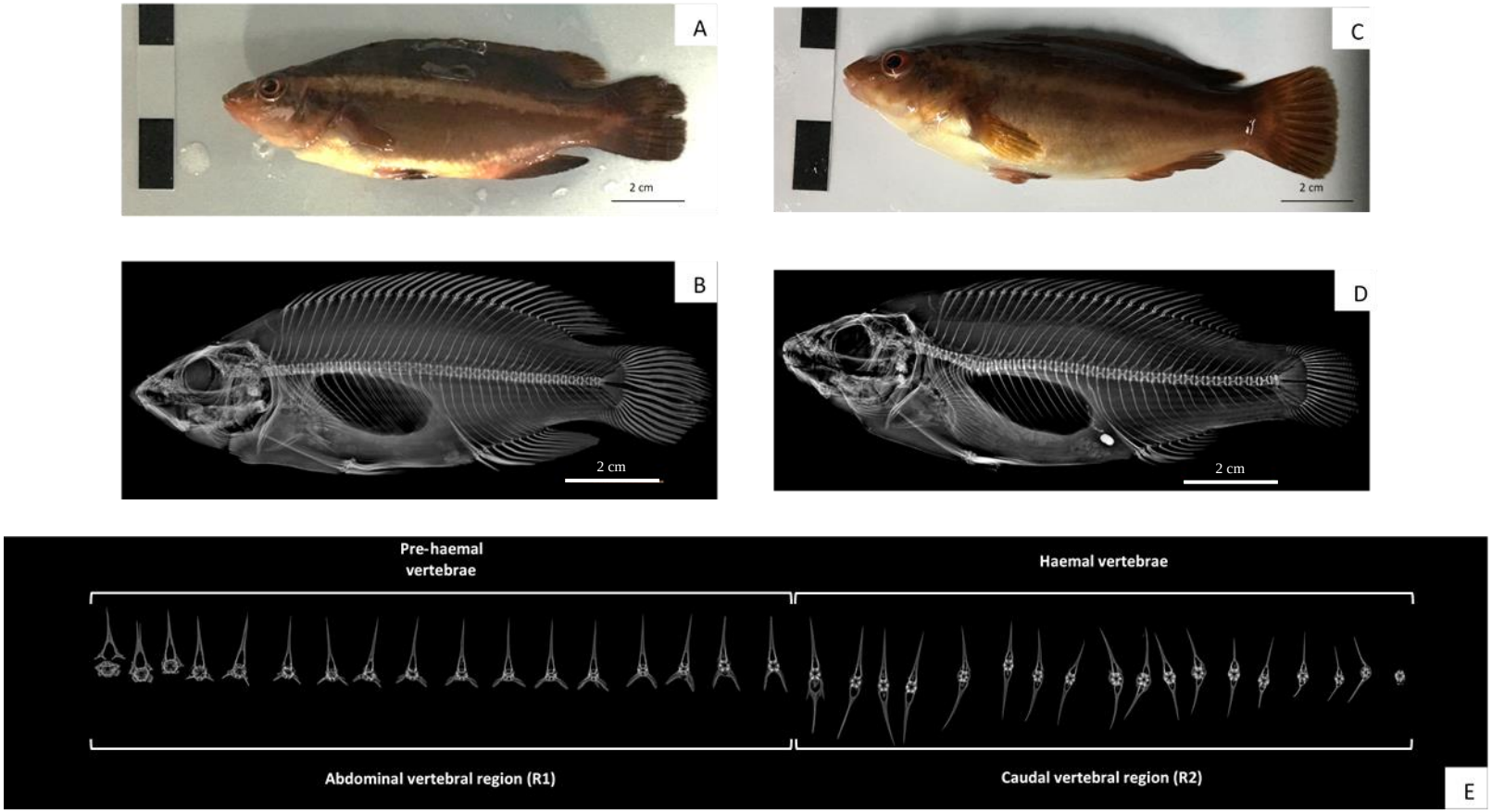
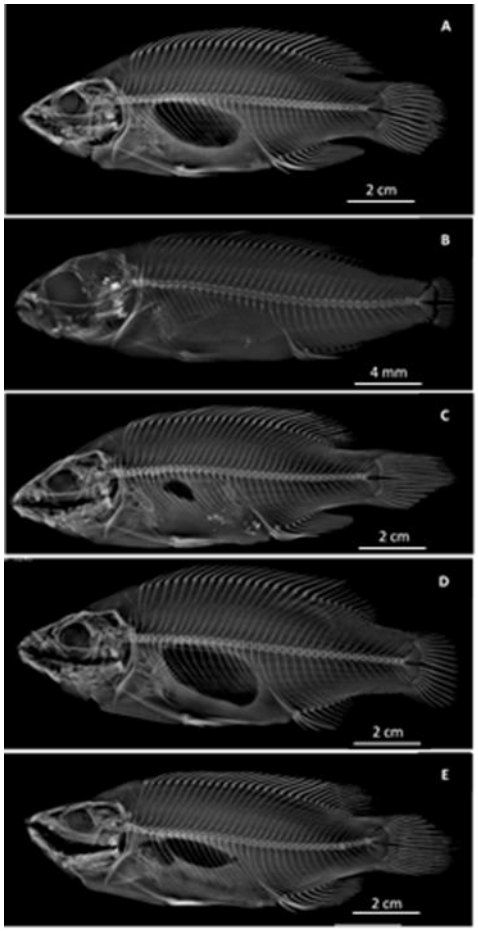

A
2 cm
C
2 cm
B
2 cm
D
2 cm
Pre-haemal vertebrae
Haemal vertebrae
Abdominal vertebral region (R1)
Caudal vertebral region (R2)
E

Figure 3



763 **Figure 4.**

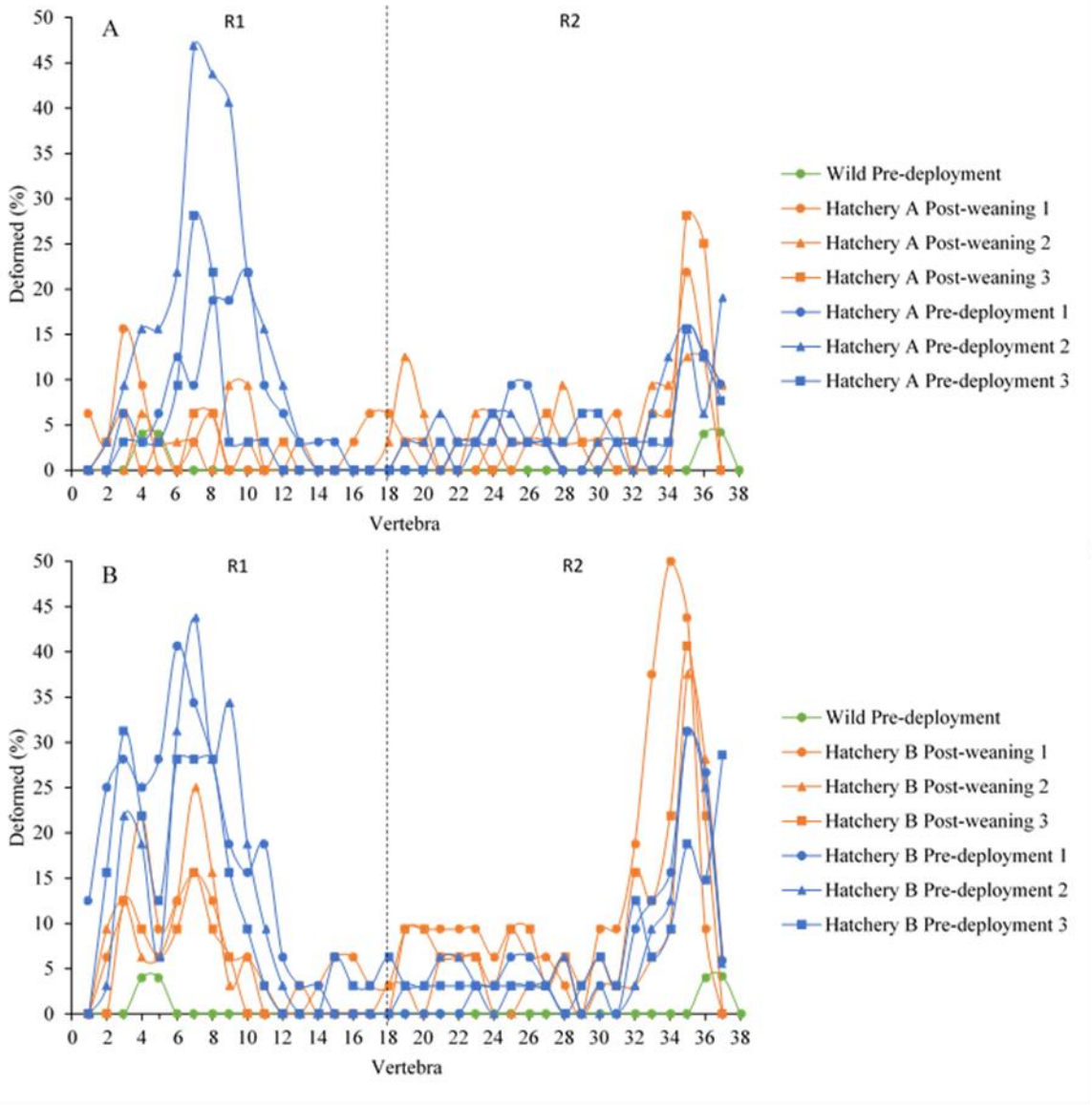


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Figure 5.

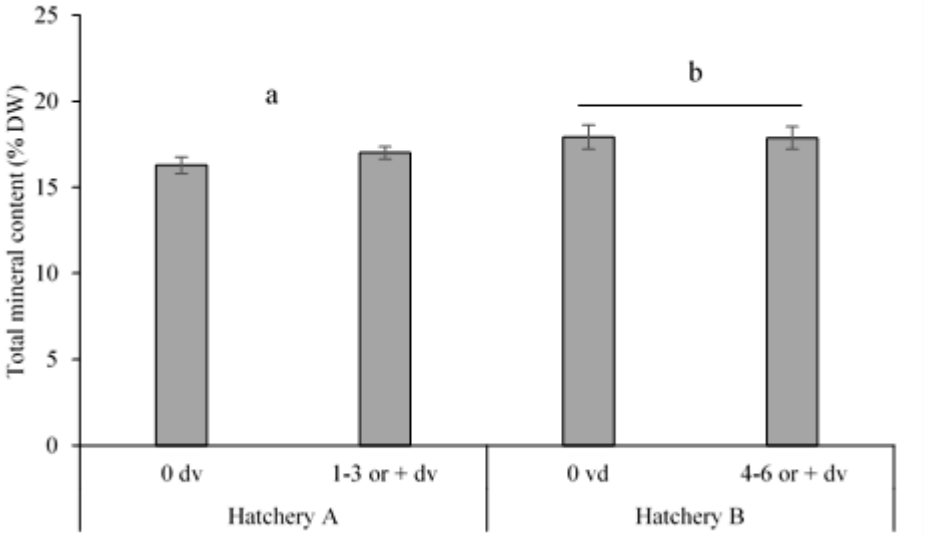
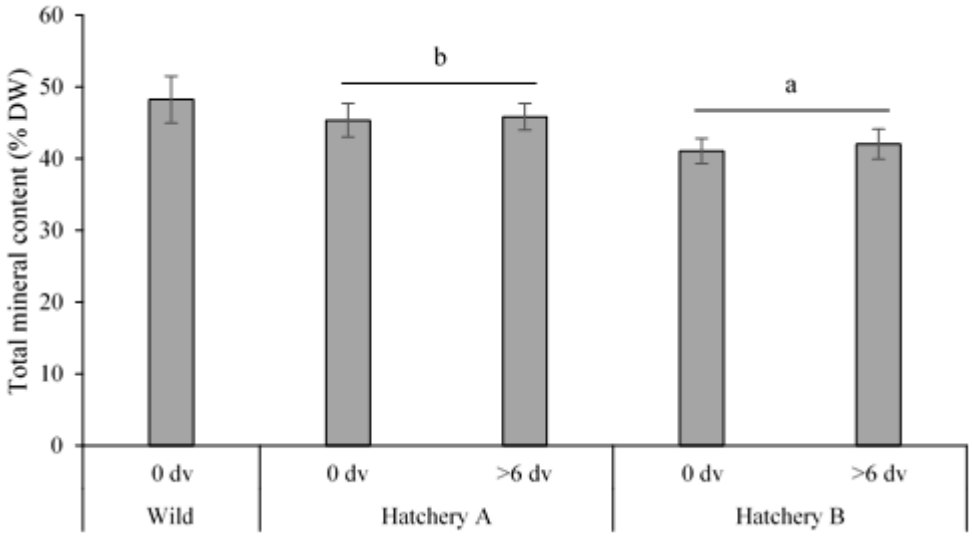

Total mineral content (% DW)
25
20
15
10
5
0
a
b
0 dv
1-3 or + dv
0 vd
4-6 or + dv
Hatchery A
Hatchery B

Figure 6.


Total mineral content (% DW)
60
50
40
30
20
10
0
b
a
0 dv
0 dv
>6 dv
0 dv
>6 dv
Wild
Hatchery A
Hatchery B