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2 **Pre-deployment acclimatisation of farmed ballan wrasse (*Labrus bergylta*) to sea-cage**  
3 **conditions promotes behaviour analogous to wild conspecifics when used as cleaner fish**  
4 **in Atlantic salmon (*Salmo salar*) farms**

5

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19

20 **Abstract**

21 Ballan wrasse (*Labrus bergylta*) are used as cleaner fish in commercial Atlantic salmon  
22 farming to remove ectoparasitic sea lice. While the delousing performance of wild wrasse is  
23 usually good, that of farmed wrasse is variable, possibly because of different conditions in  
24 hatcheries and sea cages. In this study, three passive-acoustic telemetry (PAT) trials were  
25 conducted at a salmon farm to compare the behaviour of wild and farmed wrasse and test the  
26 effect of acclimatising farmed wrasse to sea-cage conditions before deployment. Up to 40  
27 fish were monitored simultaneously for 60–124 days by triangulating tag positions within a  
28 hydrophone array every 6–10 secs. This data was used to assess fish depth and preferred cage  
29 locations and calculate activity, orientation and home ranges.

30

31 Wild wrasse occupied shallower depths ( $13.3 \pm 2.4$  m) than farmed wrasse, which remained  
32 near the bottom of the cages ( $18.2 \pm 1.7$  m). Swimming activity was higher in wild wrasse  
33 with significant diurnal variations due to nocturnal quiescence on 68% of observed days.  
34 Wild wrasse rapidly developed large home ranges ( $616.8 \pm 110.1$  m<sup>2</sup>), preferring cage  
35 corners. Hatchery-and-sea-cage acclimatisation improved the behaviour of farmed wrasse;  
36 they rapidly moved up the water column ( $9.08 \pm 2.05$  m after 1 week) and established home  
37 ranges ( $514.3 \pm 146.6$  m<sup>2</sup>), and they developed diurnal activity patterns, which may indicate a  
38 positive response to acclimatisation.

39

40 Acclimatising farmed ballan wrasse to sea-cage conditions positively improved and  
41 encouraged behaviours similar to those seen in wild wrasse, including diurnal rhythms and  
42 the establishment of home ranges, and is recommended for all farmed wrasse prior to  
43 deployment to improve delousing performance.

44

45 **Keywords:** Passive-acoustic telemetry, cleaner fish, biological control, acclimatisation, sea  
46 lice, salmonid aquaculture

47

## 48 **1 INTRODUCTION**

49 In North Atlantic salmon-farming regions, the use of cleaner fish as a component of  
50 integrated pest management strategies against the salmon lice *Lepeophtheirus salmonis* and  
51 *Caligus* spp. has increased significantly in the past decade. Two species of cleaner fish are  
52 currently being farmed, ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*).  
53 Norway is the biggest producer of cleaner fish with 30.6 million farmed in 2018 (63% of total  
54 deployed, Norwegian Directorate of Fisheries,  
55 [www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse](http://www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse)), while  
56 UK production levels in 2018 were 103,000 and 2,753,000 for ballan wrasse and lumpfish,  
57 respectively (Munro and Wallace, 2018). Securing a sustainable supply of farmed cleaner  
58 fish and becoming self-sufficient is a key priority in meeting the demands of the industry.

59

60 While reaching this target may be achievable given the current pace of progress in cleaner  
61 fish research and infrastructure investment, it is necessary to ensure that the farmed cleaner  
62 fish are robust and perform effectively once deployed at sea through good health and welfare.  
63 Indeed, improving the survival and delousing performance of farmed cleaner fish may allow  
64 their stocking densities to be reduced while maintaining optimal delousing, thereby reducing  
65 the number of fish required. However, while wild wrasse are effective delousers in  
66 commercial salmon sea cages (Treasurer, 2002), as are farmed wrasse in experimental tank  
67 studies (Leclercq et al., 2014a), the performance of farmed wrasse in sea cages has been  
68 questionable. It is possible that the anecdotal reports of variable performance of farmed  
69 wrasse in sea cages, in contrast to their effective performance in tank studies (Leclercq et al.,

70 2014a), may be due to the environmental conditions experienced by the fish in sea cages. In  
71 hatcheries, ballan wrasse are fed pelleted feeds in tanks, under artificial light regimes and at a  
72 controlled temperature, whereas sea-cage volumes are much larger with open boundaries, the  
73 fish are subject to ambient conditions, e.g. seasonal day/light and temperature regimes and  
74 tidal cycles, and supplementary feed is often provided in the form of feed blocks (Leclercq et  
75 al., 2015). Furthermore, the presence of large salmon within the sea cage may act as a  
76 stressor to farmed ballan wrasse, which have had no previous exposure to other fish species.

77

78 While the behaviour of wild and farmed ballan wrasse have not previously been compared  
79 and sea-cage acclimatisation has not previously been tested, in other species, particularly in  
80 salmonids, the behaviour of fish reared in a hatchery has been proven to be very different to  
81 wild fish. Hatchery reared fish are more aggressive at higher densities (Fenderson and  
82 Carpenter, 1971), more prone to predation (Jackson and Brown, 2011) and less successful at  
83 foraging (Brown and Laland, 2002). However, acclimatisation and conditioning regimes  
84 during the hatchery phase have been shown to improve foraging behaviour (Brown et al.,  
85 2003), improve predator response (Jarvi and Uglem, 1993) and decrease stress (Näslund et  
86 al., 2013).

87

88 Delousing performance in cleaner fish is difficult to assess in sea cages: routine lice counts  
89 on salmon provide an estimate of the lice population within each sea cage, but this can be  
90 influenced by factors such as environmental conditions, lice population dynamics and  
91 implemented lice management methods (Brooker et al., 2018a); individual cleaner fish can be  
92 sacrificed and the lice in their guts counted, but routine destructive sampling depletes the  
93 valuable stock over time. Passive-acoustic telemetry (PAT) has been proven as an effective  
94 method for observing the fine-scale activity of individual cleaner fish in salmon sea cages

95 (Leclercq et al., 2018) and was used in the present study to quantify the swimming behaviour  
96 of ballan wrasse in sea cages as a proxy for delousing behaviour. While the overall  
97 performance of the cleaner fish population in each sea cage determines their efficacy against  
98 the sea lice levels within the farm, the behaviour of each fish contributes to the performance  
99 of this population. Therefore, observing the behaviour of individual sentinel fish allows an  
100 estimate of the range of behavioural phenotypes within a population and improves our  
101 understanding of the requirements of the fish, encouraging good husbandry practices to  
102 improve welfare.

103

104 The aims of this study were to (1) compare the behaviour of wild-caught and farmed ballan  
105 wrasse in sea cages at a commercial Atlantic salmon farm, and (2) investigate the impact of  
106 sea-cage acclimatisation on the behaviour of farmed ballan wrasse.

107

## 108 **2 MATERIALS AND METHODS**

### 109 2.1 Study site

110 The investigation was performed at a commercial Atlantic salmon sea farm comprised of  
111 three groups of four sea cages (56.69 °N, 5.14 °W, Loch Leven; Mowi Scotland Ltd, UK).

112 The study used two adjacent sea cages within one group of four floating square steel  
113 platforms (HP 2000, Wavemaster, AKVA Group, Inverness, Scotland) each holding a  
114 double-sized net bag (24 × 24 m square; 15 m (at edges) to 20 m (at centre) depth inverted  
115 pyramid; 18 mm mesh) for *in-situ* biofouling control by switching the net bag and air-drying.

116

117 Atlantic salmon were fed a commercial extruded diet (BioMar (UK) Ltd) to visual satiation  
118 twice daily using surface rotor spreaders and underwater video monitoring (Akvasmart CCS  
119 feed system, SmartEye 360 twin camera; AKVA Group). Two sinking wrasse hides (1 m Ø

120 weighted ring; 2 m high; plastic fake kelp; Leclercq et al., 2018) were suspended from a rope  
121 at opposite corners of each cage at 8–12 m depth (Fig. 1), and wrasse feed blocks suspended  
122 from ropes were offered weekly adjacent to the hides (600 g blocks, n = 2 / cage; Leclercq et  
123 al., 2015). Mean sea lice counts were obtained weekly on 10 salmon/cage. Salmon and  
124 cleaner fish mortalities were removed daily using hand nets and an air-lift pump connected to  
125 a bottom collector (LiftUP Akva AS, Eikelandsosen, Norway); no significant mortality  
126 events occurred during the study.

127

## 128 2.2 Acoustic telemetry system

129 A PAT system (HTI-Vemco Inc., Seattle, WA, USA) was used to record the positions of  
130 acoustic-tagged ballan wrasse during each trial. The acoustic tags (795 LD; 6.8 × 20.0 mm;  
131 0.55 g in water) emit at a single frequency (307.2 kHz) and each one is programmed with a  
132 unique, user-defined pulse rate interval (PRI) to allow tag identification (Ehrenberg and  
133 Steig, 2009). The 3D positioning of each tag pulse (up to 20 cm resolution) is achieved by  
134 measuring the time delay to at least four hydrophones and triangulating its position. Before  
135 the study commenced, an array of eight underwater hydrophones (Model 590, omni-  
136 directional) was deployed around the perimeter of both experimental sea cages as described  
137 by Leclercq et al. (2018) (Fig. 1).

138

## 139 2.3 Experimental fish

140 Three trials using PAT were conducted at the site: (1) a comparison of wild-caught *vs.*  
141 farmed ballan wrasse in 2015, (2) an investigation of the effect of (a) hatchery acclimatisation  
142 and (b) hatchery-and-sea-cage acclimatisation on farmed ballan wrasse survival and  
143 behaviour in 2016 (Table 1).

144

145 Prior to the start of the trial 1, the experimental cages were stocked in October 2014 with Q2  
146 2014 Atlantic salmon, and when the trial commenced, the cage contained approximately  
147 34,600 salmon with a mean weight of 3.42 kg. Wild labrids captured from Arisaig, West  
148 Coast of Scotland (n = 3,200, 7.2 % of salmon stock at time of deployment) were deployed  
149 into the cage, and the deployed population was comprised of ballan wrasse (57.9 %),  
150 goldsinny wrasse (*Ctenolabrus rupestris*; 29.9 %), corkwing wrasse (*Crenilabrus melops*, 7.6  
151 %), rockcook wrasse (*Centolabrus exoletus*, 3.7 %) and cuckoo wrasse (*Labrus mixtus*, 0.9  
152 %). Prior to the start of the experiment, the mean mortality in the wild wrasse population was  
153 0.0001% per day. For tag implantation, wild ballan wrasse were captured from the  
154 experimental cage using non-baited creel pots deployed at 6–12 m depth, slowly raised to the  
155 surface (~2 m / min). Farmed ballan wrasse for trial 1 were reared at Otter Ferry Seafish Ltd.  
156 (Tighnabruaich, Argyll, Scotland), where they were hatched in 2013 from wild-caught  
157 broodstock. Fish were reared in 2.8 m<sup>3</sup> circular flow-through tanks (salinity 33–34 ppt) under  
158 ambient temperature, 24 h light, and fed with pelleted feed (BioMar Symbio 2 mm). Fifty  
159 fish were transported in a tank via road to the Loch Leven site and retained in a 300L  
160 perforated plastic barrel submerged below a floating jetty prior to tag implantation.

161

162 Prior to the start of trial 2, the experimental cages were stocked in June 2016 with Q1 2016  
163 Atlantic salmon, and when the first acclimatisation trial commenced, the two cages contained  
164 approximately 56,700 and 41,600 salmon with mean weights of 461.5 g and 453.9 g. Farmed  
165 ballan wrasse for trial 2 originated from Otter Ferry Seafish Ltd., where they were hatched in  
166 2014 from wild-caught broodstock. For the first acclimatisation trial (2a), 1,620 wrasse were  
167 maintained in standard rearing conditions (non-acclimatised group, 24 h light regime, BioMar  
168 Symbio 2 mm pelleted feed, no tank furniture), and 1,950 wrasse were acclimatised for  
169 around one month (11<sup>th</sup> May – 19<sup>th</sup> June, 2016), both in 2,000 L tanks.

170

171 The hatchery acclimatisation involved changing the environmental rearing conditions:  
172 artificial kelp hides were present in the tank, a simulated natural photoperiod was provided  
173 via skylights in the roof of the tank room, and supplementary feed blocks were provided in  
174 the tanks (Leclercq et al., 2015) in addition to pelleted feed (Biomar Symbio 2.0 mm). After  
175 two weeks, the pelleted feed was withdrawn. The mean water temperature during this period  
176 was  $11.5 \pm 0.5$  °C, and the mean weight of the fish was 38 g at the start of the acclimatisation  
177 period. Following the hatchery acclimatisation, both groups of fish were transported in tanks  
178 via road to the Loch Leven site. Acclimatised fish were stocked into one cage (n = 1,950; 3.4  
179 % of salmon stock) and non-acclimatised fish were stocked into another (n = 1,620; 3.9 % of  
180 salmon stock) on 11<sup>th</sup> June 2015. At the time of stocking 50 fish per group were retained in  
181 300L perforated plastic barrels submerged below a floating jetty for subsequent acoustic tag  
182 implantation.

183

184 For the second acclimatisation trial (2b), 200 wrasse from the same cohort of fish used in trial  
185 2a were retained at the hatchery under standard rearing conditions as before. Six weeks prior  
186 to the start of the trial, half of the wrasse were acclimatised for around one month (5<sup>th</sup>–30<sup>th</sup>  
187 August, 2016) using the same conditions as for trial 2a. The mean water temperature during  
188 this period was  $12 \pm 0.5$  °C, and the mean weight of the fish was 40 g at the start of the  
189 acclimatisation period. Two weeks prior to the start of the trial (30<sup>th</sup> August 2016), these fish  
190 were transported to the Loch Leven site as before and introduced to a 2m x 2m x 4m keep net  
191 within the sea cage. The keep net contained artificial kelp hides, and supplementary feed  
192 blocks were offered. For tag implantation, these fish were captured using a hand net. Two  
193 days prior to the start of the trial (11<sup>th</sup> September 2016), the 100 remaining non-acclimatised



194 wrasse were transported to the Loch Leven site and retained in submerged, perforated 300L  
195 plastic barrels ready for tag implantation and stocking into the cages.

196

197 Water temperature, salinity and dissolved oxygen were measured at 30 min intervals at 1, 4, 8  
198 and 12 m depth in 2015 (trial 1) and 1, 2, 4 and 12 m depth in 2016 (trials 2a and 2b) over the  
199 study duration (see supplementary data) using data loggers (HOBO U24-002C; HOBO U26-  
200 001; Onset Computer Corporation, Bourne, MA, USA) attached to a weighted line deployed  
201 between the experimental cages.

202

#### 203 2.4 Surgical procedure and tagged fish deployment

204 Before each trial, each acoustic tag was programmed with a unique PRI ranging from 6,275  
205 to 9,957 msec using an acoustic tag programmer (490-LP, HTI-Vemco (USA) Inc., Seattle,  
206 WA, USA) connected to a Windows laptop running TagProg software (v6.0, HTI-Vemco  
207 (USA) Inc., Seattle, WA, USA) and stored in a mild antiseptic solution (0.5g/L, Presept;  
208 Johnson & Johnson, CA, USA). Tags were implanted into the coelemic cavity of the fish  
209 following the surgical procedure described in Leclercq et al. (2018). Following recovery, fish  
210 were isolated at sea in 300L perforated barrels for 48 h with any spare fish not tagged, then  
211 examined for survival and suture integrity prior to release in their designated sea cage along  
212 with any spare fish. No mortalities occurred during the recovery period before deployment in  
213 the sea cages. Once deployed, tag signals from tagged fish were recorded continuously until  
214 the tag batteries expired after several months. Hide tags (n = 4; Model 795 LG; 11.0 x 25.0  
215 mm) were programmed at 9,313–15,235 msec and one was deployed within each cleaner-fish  
216 hide to track the movements of the hides resulting from tidal currents. All experiments were  
217 carried out in accordance with the Animal (Scientific Procedures) Act 1986 UK and were  
218 approved by the University of Stirling animal welfare ethical review board.

219

## 220 2.5 Data processing

221 Raw acoustic data were processed to identify tags and calculate their positions using  
222 MarkTags (v6.10) and AcousticTag (v6.10) software (HTI-Vemco (USA) Inc., Seattle, WA,  
223 USA), which were saved as hourly Microsoft Access (.mdb) database files consisting of a list  
224 of tag number and Coordinated Universal Time (UTC) stamp with three-dimensional  
225 Cartesian coordinates (x, y, z). Specifically, a noise filter was used to extract individual tag  
226 signals according to their PRIs, and then a 3D algorithm was used to calculate the position of  
227 each individual tag pulse within the study coordinate system based on simultaneous  
228 detections from at least four hydrophones. Up to 16,023 positions fish/day were recorded but  
229 where fish remained in hides or on the bottom of the sea cage, many signals were blocked  
230 and the number of detected positions was as low as 1,082 fish/day. The hourly position files  
231 were converted to comma separated values (.csv) files, and all further analyses were  
232 conducted in R (R Foundation for Statistical Computing, [www.R-project.org/](http://www.R-project.org/)).

233

234 Hourly files were merged into daily files, which were further processed and filtered as  
235 follows: the time between detections was used to calculate the minimum swimming speed  
236 (BL (body lengths)/sec) between successive detections based on the length of each fish at the  
237 time of tag implantation; headings (degrees) were calculated based on each successive set of  
238 detections; spurious tag detections resulting from attenuation or reflection of the tag signal  
239 were removed by filtering out all subsequent detections with a time delay  $< 5$  sec (all tag  
240 PRIs were  $> 5$  sec) and using a moving average fish location filter to eliminate false  
241 detections; each individual fish track was visualised and analysed to identify any moribund or  
242 dead fish (date and time of mortality was noted), and tag detections emitted from dead fish  
243 were removed from the dataset.

244

245 The length of the datasets from each trial were selected based on numbers of tagged fish  
246 mortalities and spent tags (due to tag batteries being drained). Fish were selected for  
247 inclusion in the final datasets based on tags emitting signals for the entire period of the  
248 dataset and fish remaining alive for at least 10 days after the final day of the selected length  
249 of each dataset.

250

251 Solar and tidal status were assigned to each tag detection based on their time stamp as  
252 follows: solar status (dawn, day, dusk, night) was based on sunrise and sunset times at Fort  
253 William, Scotland (<http://www.timeanddate.com/sun/uk/fort-william>, 9 miles north of study  
254 site) with a 2-h dawn/dusk period centred on sunrise/sunset and night/day starting/ending 2 h  
255 before/after sunrise/sunset; tidal status was based on tidal charts for Loch Leven Head,  
256 Scotland (<http://www.tidetimes.co.uk/loch-leven-head-tide-times>, 5.6 miles east of study site)  
257 (high- and low-tides (slack tide) were defined as a 20-min time period centred at each  
258 predicted tidal maximum/minimum, and similarly, mid-tides (flood and ebb tides) were  
259 defined as a 20-min time period centred between successive high and low tides); tag  
260 detections during dawn and dusk periods were excluded from analyses as they are transition  
261 periods between light and dark.

262

263 Based on their Cartesian coordinates, each tag detection was categorised into one of five  
264 locations within each cage: bottom (below 15 m), hide corner ( $6 \times 6$  m, 0–15 m depth at each  
265 cage corner where hides were present), empty corner ( $6 \times 6$  m, 0–15 m depth at each cage  
266 corner where no hides were present), edges (outside the theoretical cage volume or within 6  
267 m inside the cage edges, corners excluded, 0–15 m depth), centre ( $13 \times 13$  m at the cage  
268 centre; 0–15 m depth) and hides (2 m diameter  $\times$  3 m depth cylinder centred to each shelter

269 location using hide tag positions). A degree of net-bag distortion from tidal flow occurred,  
270 which resulted in some tag detections outside the theoretical cage volume.

271

272 Home ranges for each fish were estimated from bivariate normal fixed kernel utilisation  
273 distributions (KUDs), which were calculated over a  $0.5 \times 0.5$  m resolution grid using the  
274 *adehabitatHR* package in R, and the 95% KUD and 50% KUD areas were termed home  
275 range and core area, respectively (March et al., 2010). Changes in home ranges were  
276 investigated by plotting the daily cumulative KUD<sub>95</sub> for each fish over the period of the  
277 study. The home range of each fish is established when the asymptote is reached, which was  
278 defined as the day that the change in KUD<sub>95</sub> between two consecutive days was less than 5%  
279 (Rechisky and Wetherbee, 2003).

280

## 281 2.6 Statistical analysis

282 Due to the large-scale nature of the study conducted at a commercial salmon farm, it was not  
283 possible to use replicate treatment groups (cages). However, individual fish were treated as  
284 pseudo replicates for all analyses. Means of individual units (fish) were checked for  
285 normality using the Anderson-Darling test and for homogeneity of variance using Levene's  
286 test and observations of residual plots. Log transformations were used where necessary to  
287 normalise data where possible. A one-way analysis of variance (ANOVA) was used to  
288 compare differences in means between groups, and a repeated-measures ANOVA was used  
289 to investigate the effect of daily changes in depth or activity according to the time of day with  
290 Tukey post-hoc tests used for pairwise comparisons. The Chi-squared goodness-of-fit test  
291 was used for nominal frequency data (i.e. fish headings), but due to the very large datasets,  
292 all comparisons were highly significant ( $P < 0.001$ ) even where differences were not  
293 biologically important (Anderson et al., 2000). Consequently, Cramer's V-test (*rcompanion*

294 package in R) was used to measure effect size with magnitudes defined as small =  $> 0.042$ ,  
295 medium =  $> 0.127$  and large =  $> 0.212$  (Cohen, 1988) where the number of categories  $k = 8$   
296 ( $45^\circ$  per category). To compare trends in depth over time between groups of fish, general  
297 linear models were fitted to daily means for each group using least-squares regression, and  
298 significant differences between slopes (trends) were estimated using pairwise comparisons  
299 (*lsmeans* package in R).

300

### 301 **3 RESULTS**

302 The datasets used for analyses all commenced on the deployment of the tagged fish and  
303 ended 43, 30 and 30 days after deployment for trial 1, trial 2a and trial 2b, respectively.

304 During trial 1, no data was collected for a total of six days due to temporary network failures.

305 Water quality data are summarised for each trial in Table 2, and time-series plots are

306 provided as supplementary data. On day 7 of trial 1, a hydrogen peroxide bath treatment was

307 administered to treat against amoebic gill disease, which affected the behaviour of the wrasse

308 for several days.

309

#### 310 3.1 Mortality

311 Mortality in trial 1 was 11.1% for wild wrasse (2 mortalities) and 33.3% (7 mortalities) for

312 farmed wrasse over 43 days (Fig. 2). In addition, a further one farmed wrasse and two wild

313 wrasse tags ceased emitting during the study (presumed batteries expired) meaning that 14

314 wild wrasse and 10 farmed wrasse were included in the analyses of trial 1.

315

316 Mortality in trial 2a was 15% for acclimatised wrasse (3 mortalities) and 10% for non-

317 acclimatised wrasse (2 mortalities) over 30 days (Fig. 2). In addition, a further four tags from

318 each group ceased emitting during the study (presumed batteries expired) meaning that 13  
319 acclimatised wrasse and 14 non-acclimatised wrasse were included in the analyses of trial 2a.

320

321 Mortality in trial 2b was 5.9% (1 mortality) in acclimatised wrasse and 0% in non-  
322 acclimatised wrasse over 30 days (Fig. 2). However, a further four mortalities in the  
323 acclimatised fish and two mortalities in the non-acclimatised fish were seen in the 10 days  
324 following the 30 days that were analysed, so these fish were also removed from the dataset.  
325 In addition, two tags in acclimatised fish and one tag in a non-acclimatised fish ceased  
326 emitting during the study period, meaning that 10 acclimatised fish and 14 non-acclimatised  
327 fish were included in the final dataset for trial 2b.

328

### 329 3.2 Sea lice numbers

330 Sea lice numbers on the salmon in the experimental cage remained low throughout trial 1,  
331 with the maximum mean numbers recorded being 0.32 lice/fish for all motile lice stages and  
332 0.2 lice/fish for gravid females (data not shown). As both groups of fish (wild and farmed)  
333 were deployed into the same sea cage, it was not possible to determine the level of delousing  
334 of either group of fish, although the low lice numbers suggest that delousing did occur.

335

336 During trial 2a, lice numbers increased from 0.2 and 0.1 lice/fish (all motile stages) to 1.65  
337 and 3.4 lice/fish at the beginning of July 2016 (day 17 of the trial) for acclimatised and non-  
338 acclimatised cages, respectively (data not shown). The mean number of gravid female lice  
339 was very low at 0.05 lice/fish or lower throughout the trial in both cages. Following the  
340 administration of medicated feed on 12–16<sup>th</sup> July, the number of lice decreased to zero.

341

342 During trial 2b, the lice numbers fluctuated between 0.65 and 3.2 lice/fish in the acclimatised  
343 wrasse cage and 2.2 and 5.2 lice/fish in the non-acclimatised wrasse cage (all motile stages)  
344 (data not shown). The number of gravid female lice was consistently 0.1 or less in the  
345 acclimatised wrasse cage and 0.5 or less in the non-acclimatised wrasse cage.

346

### 347 3.3 Depth

348 In trial 1, the mean depth of wild wrasse for the duration of the study was significantly  
349 shallower than farmed wrasse ( $13.3 \pm 2.4$  m vs.  $18.2 \pm 1.7$  m, respectively,  $F = 173.6$ ,  $P = 0$ ,  
350 Fig. 3a), although there were no significant differences between daily daytime and night time  
351 mean depths in either group (Fig. 3b & 3c). In the night following the bath treatment, the  
352 mean depth of the wild fish increased dramatically to  $19.8 \pm 0.6$  m, but decreased gradually  
353 to pre-treatment levels in the following days, whereas the farmed fish remained deep  
354 throughout the study. Over the course of the trial, the daytime mean depth of wild wrasse  
355 decreased from  $14.03 \pm 1.04$  m on day 1 to  $11.91 \pm 1.44$  m on day 43 (Fig. 3b) whereas the  
356 daytime mean depth of farmed wrasse increased from  $14.02 \pm 0.97$  m on day 1 to  $18.71 \pm$   
357  $1.17$  m on day 43 (Fig. 3c); slopes of fitted linear regressions for wild and farmed wrasse  
358 depth over time were significantly different ( $t$ -ratio =  $-3.718$ ,  $P = 0.0004$ ).

359

360 In trial 2a, there was no significant difference in depth preferences between both hatchery-  
361 acclimatised and non-acclimatised fish ( $13.8 \pm 1.7$  m vs.  $13.3 \pm 1.8$  m, respectively,  $F =$   
362  $2.061$ ,  $P = 0.15$ , Fig. 3a), and there were no significant differences between daytime and night  
363 time daily mean depths (Fig. 3d & 3e). Although the mean depths of both groups of fish  
364 decreased slightly during the study, the difference in mean depths between the start and end  
365 of the trial and the slopes of fitted linear regressions were not significantly different ( $t$ -ratio =  
366  $1.577$ ,  $P = 0.12$ ).

367

368 At the start of trial 2b (day 1), both acclimatised and non-acclimatised fish remained deep  
369 ( $15.2 \pm 2.3$  m vs.  $19.1 \pm 0.4$  m, respectively) (Fig. 3f & 3g). However, the acclimatised fish  
370 rapidly decreased their depth; by day 7 their mean daytime depth was  $9.08 \pm 2.05$  m, and they  
371 remained relatively high ( $>11$ m) in the water column for the rest of the trial. In comparison,  
372 the non-acclimatised fish were slower to swim up in the water column and the change in  
373 depth was less, although the slopes of fitted linear regressions for acclimatised and non-  
374 acclimatised fish were not significantly different from each other ( $t$ -ratio = 1.577,  $P = 0.12$ ).  
375 Overall, however, non-acclimatised fish were significantly deeper than acclimatised fish ( $9.8$   
376  $\pm 2.0$  m vs.  $13.7 \pm 1.9$  m, respectively,  $F = 188.6$ ,  $P = 0$ , Fig. 3a), although there were no  
377 significant differences in either group between daytime and night time daily mean depths.

378

379 In all trials, there was a large variation in the mean depths of individual fish although  
380 variations in the daily means of individual fish were generally low. In all trials, only two wild  
381 fish and one hatchery-and-cage-acclimatised fish showed a significant difference between  
382 daytime and night time mean daily depths (S4a & S4e).

383

#### 384 3.4 Activity

385 In trial 1, the mean daytime activity of wild fish was significantly higher than their mean  
386 night time activity ( $0.39 \pm 0.08$  BL/s vs.  $0.26 \pm 0.04$  BL/s, respectively,  $F = 159.7$ ,  $P = 0$ , Fig.  
387 4a), whereas the same was not significantly different in farmed fish ( $F = 3.119$ ,  $P = 0.08$ ).  
388 Furthermore, the mean daytime activity of wild fish was significantly higher than mean  
389 daytime and night time activity in farmed fish ( $F = 41.88$ ,  $P = 0$  and  $F = 47.02$ ,  $P = 0$ ,  
390 respectively, Fig. 4a). When comparing daily means, wild fish daytime activity was



391 significantly higher than night time activity on 68% of observed days, as opposed to 16% of  
392 observed days in farmed fish (Fig. 4b, 4c).

393

394 In trial 2a, the hatchery acclimatisation had no significant effect on swimming activity (Fig.  
395 4a). When comparing daily means, daytime and night time activity was significantly different  
396 in only one day in each group (Fig. 4d, 4e).

397

398 In trial 2b, mean daytime activity was significantly higher than mean night time activity in  
399 hatchery-and-cage-acclimatised fish ( $0.65 \pm 0.09$  BL/s vs.  $0.48 \pm 0.06$  BL/s, respectively,  $F =$   
400  $63.47$ ,  $P = 0$ , Fig. 4a), but not in non-acclimatised fish ( $F = 3.39$ ,  $P = 0.07$ ). Due to the wide  
401 variation of daytime activity between fish in hatchery-and-cage-acclimatised fish, their mean  
402 daytime activity was not significantly different from non-acclimatised fish in trial 2b (Fig.  
403 5a). Furthermore, the large variability between fish means that daily daytime activity was  
404 only significantly different to daily night time activity in 23% of observed days ( $P < 0.05$ –  
405  $0.01$ , Fig. 4f).

406

407 In all trials, there was a clear distinction between individual fish with a significantly higher  
408 mean daytime activity than mean night time activity and those with very similar activity at  
409 different times of day. In trial 1, 86% of wild fish had significantly higher swimming activity  
410 during the day compared to the night ( $P < 0.001$ , Fig. 5a) with the increase ranging from 0.07  
411 to 0.23 BL/s. Conversely, mean activities were low in trial 1 farmed fish ( $0.27 \pm 0.01$  –  $0.39$   
412  $\pm 0.05$  BL/s) and only 30% exhibited a significant difference between mean daytime and  
413 night time activity, and this increase was notably lower at 0.04 BL/s (Fig. 5b). In trial 2a,  
414 swimming activity was higher during the day compared to the night in only two hatchery-  
415 acclimatised fish (Fig. 5c), whereas 70% of hatchery-and-cage-acclimatised fish (trial 2b) had

416 significantly elevated daytime activity compared to 15% in non-acclimatised fish (trial 2b)  
417 with the increase in activity ranging from 0.06 to 0.39 BL/s and 0.12 to 0.26 BL/s,  
418 respectively (Fig. 5e, 5f).

419

### 420 3.5 Cage locations

421 During the first week of trial 1, wild wrasse spent approximately 50% of their time below  
422 15m, and this increased to 82% on day 8 following a hydrogen peroxide bath treatment  
423 administered on day 7 (Fig. 6a). On the day of the treatment, tagged fish spent a high  
424 proportion of their time in the centre of the cages compared to all other days (14.7% and  
425 14.8% for wild and farmed wrasse, respectively) due to the reduction in the volume of the sea  
426 cages for the bath treatment. For wild wrasse, the daily time spent below 15m decreased after  
427 day 8 and then remained at approximately 30–40% for the rest of the study. The edges and  
428 corners were also preferred locations, with more time spent in hide corners than empty  
429 corners, although the hides were very seldom used (data not shown). Edges and corners  
430 together accounted for approximately 50–70% of wild wrasse daily time from day 19. Visits  
431 to the cage centre were infrequent (0.38–3.52% of daily time). In comparison, farmed wrasse  
432 in trial 1 spent 60–90% of their time below 15m throughout the trial (Fig. 6b). Other  
433 preferred locations of the farmed wrasse were edges and corners with very little time spent in  
434 the cage centre.

435

436 In trial 2a, the behaviour of the hatchery-acclimatised fish and non-acclimatised fish were  
437 similar, and the acclimatisation did not appear to have a significant impact on their preferred  
438 cage locations, with the fish spending the majority of the time in the corners, particularly the  
439 hide corners (data not shown).

440

441 In trial 2b, the hatchery-and-cage-acclimatised fish spent increasingly less time below 15m  
442 during the day, from 67.3% on day 1 to 27.4% on day 5 (Fig. 6c), a pattern also reflected in  
443 their mean depths (Fig. 3e). As in the wild fish, corners and edges were preferred locations,  
444 with hide corners being preferred over empty corners, and an increasing proportion of time  
445 was spent in the centre of the cage towards the end of the trial (maximum of 12.8% on day  
446 25). In contrast, non-acclimatised fish in trial 2b spent more time during the day below 15m  
447 at the start of the trial (90.9% on day 1), which decreased gradually to 28.2% on day 11 (Fig.  
448 6d). As with the acclimatised fish, edges and corners were the preferred locations, with hide  
449 corners being preferred over empty corners.

450

451 Night time locations in all trials were similar to daytime locations, except for in wild fish and  
452 hatchery-and-cage-acclimatised fish, which regularly spent more time in the corners during  
453 the night (S5).

454

### 455 3.6 Orientation

456 Tidal flow in the sea cages at the study site is on an ESE-WNW axis, and farmed wrasse  
457 strongly oriented to this axis whereas wild wrasse showed a weak orientation with headings  
458 distributed more evenly (Fig. 7a, 7b). While both wild and farmed wrasse headings were  
459 significantly different from a uniform distribution of headings due to the large sample sizes  
460 ( $P = 0$ ), there was no sizeable effect in wild wrasse ( $V < 0.042$ ), whereas a medium-size  
461 effect was seen in farmed wrasse ( $V > 0.127$ ). In trial 2b, non-acclimatised fish were strongly  
462 oriented on an ESE-WNW axis with a small effect size ( $V > 0.042$ , Fig. 7d), whereas in  
463 hatchery-and-cage-acclimatised fish, fish headings were more evenly distributed with no  
464 sizeable difference from a uniform distribution ( $V < 0.042$ , Fig. 7c).

465

### 466 3.7 Home ranges and core areas

467 There was considerable variation in the home ranges and core areas of individual fish (Table  
468 3). Wild fish had the largest home ranges and core areas, and in most fish their home ranges  
469 covered the majority of the cage area (Fig. 8a). In contrast, farmed fish in trial 1 had the  
470 smallest home ranges and core areas, and their ranges were primarily at the centre of the cage  
471 (comparison with depths and locations indicates that these fish were below 15m). Both home  
472 ranges and core areas were significantly larger in wild fish than in farmed fish ( $F = 11.81$ ,  $P =$   
473  $0.00002$ ).

474

475 The home ranges and core areas of hatchery-and-cage-acclimatised fish in trial 2b were  
476 statistically similar to those in wild fish ( $F = 11.81$ ,  $P = 0.17$ ), and the core areas were mostly  
477 in the corners and hides (Fig. 8c). Although the mean home ranges and core areas of non-  
478 acclimatised fish in trial 2b were smaller than in acclimatised fish (404.6 and 46.1 vs. 514.3  
479 and 78.3, respectively), the populations were not significantly different ( $F = 11.81$ ,  $P = 0.15$ ).

480

481 The calculation of cumulative home ranges for individual fish shows that the home ranges of  
482 wild fish in trial 1 were larger than in farmed fish, and they were established over two days  
483 earlier on average ( $8.07 \pm 3.83$  d vs.  $10.5 \pm 5.4$  d for wild and farmed fish, respectively, Fig.  
484 9a, 9b). In trial 2, hatchery-and-cage-acclimatised fish rapidly established their home ranges  
485 in  $8.1 \pm 4.28$  d compared to  $10.9 \pm 5.02$  d in non-acclimatised fish (Fig. 9c, 9d).

486

## 487 **4 DISCUSSION**

488 Passive-acoustic telemetry has been proven as a useful technique for observing the fine-scale  
489 behaviour of individual cleaner fish in salmon sea cages (Leclercq et al., 2018), and this  
490 study successfully used PAT in commercial salmon sea cages to compare the behaviour of

491 wild and farmed ballan wrasse and investigate the effect of acclimatisation to sea-cage  
492 conditions. Hatchery acclimatisation alone had limited impact on the behaviour of the fish  
493 once deployed at sea, whereas a combination of hatchery-and-cage acclimatisation caused the  
494 fish to rapidly decrease their depth once deployed, develop diurnal rhythms in activity and  
495 establish home ranges earlier than non-acclimatised fish. These behaviours may be a proxy  
496 for delousing behaviour.

497

498 Although sea lice numbers were recorded weekly for farm records during the trials, due to the  
499 low numbers sampled, chemical treatments, natural fluctuations and the aggregation of some  
500 wrasse treatment groups within the same cage, it is difficult to draw any conclusions on the  
501 delousing performance of the wrasse from this data. Nonetheless, lice numbers in the  
502 acclimatised wrasse cage in trials 2a and 2b were generally lower than in the non-  
503 acclimatised wrasse cage, which may indicate a higher level of delousing in the acclimatised  
504 wrasse. Underwater cameras used for monitoring stocks and feeding rates may be used to  
505 corroborate acoustic data and records of lice numbers. However, consultation with the farm  
506 workers revealed that delousing behaviour had not been observed through the cameras  
507 suggesting that there is a low frequency of delousing behaviour and/or delousing behaviour  
508 does not occur near the cameras at the centre of the pens.

509

#### 510 4.1 Survival

511 The low survival of cleaner fish in commercial salmon sea cages is a key bottleneck that  
512 needs to be addressed (Brooker et al., 2018b) and more data is required from commercial  
513 farms to better understand the multifactorial causes of these mortalities (Powell et al., 2018).  
514 While only a relatively small number of fish were tagged in this study, it provides an  
515 estimation of survival rates in a commercial sea-cage environment. Fish mortalities are

516 collected routinely in salmon farms, although some of the tagged fish in these studies were  
517 not collected after death suggesting that actual mortality rates may be higher than predicted.  
518 Mortality was low to moderate in all trials (0.26–0.5% per day) and relatively consistent for  
519 the duration of the trials, except for farmed wrasse in trial 1, where mortality was 33% over  
520 43 days or 0.78% per day. This high mortality may be due to the smaller size of the farmed  
521 fish when deployed, which was further confounded by the bath treatment seven days post-  
522 deployment. It is not possible to identify the causes of mortality, and the tagging procedure  
523 may have caused some, especially in the period following surgery and release of the fish into  
524 the sea cages. In all three trials, however, no mortalities were seen in the 48 h following  
525 surgery before they were released into the sea cages, and there was no peak in the rates of  
526 mortality after the surgery, suggesting that few, if any, mortalities were due to the tagging  
527 procedure. The two mortalities in wild wrasse were caused by the fish being trapped in the  
528 net during a net change, although this did not occur in the 2016 trials, suggesting that this  
529 problem was identified and eliminated. However, it highlights the importance of careful  
530 observation during net changes to avoid unnecessary cleaner fish mortalities.

531

#### 532 4.2 Wild vs. farmed wrasse behaviour

533 Wild ballan wrasse are effective salmon delousers in salmon sea cages (Treasurer, 2002,  
534 2013; Brooker et al., 2018b). Although farmed ballan wrasse are proven to be very efficient  
535 delousers in tank trials (Leclercq et al., 2014a), it has been suggested from farm observations  
536 that they may not always perform effectively in sea cages, which could be due to their  
537 hatchery rearing and sea-cage deployment environments being very different. Skiftesvik et al.  
538 (2013) found that farmed wrasse were as effective as wild wrasse at delousing in sea cages,  
539 but these were in small experimental cages, which are different to full-size commercial sea  
540 cages. Therefore, if the behaviour of the wild wrasse in the current study is typical of wild

541 wrasse behaviour in sea cages following a suitable period of acclimatisation, it can be used as  
542 a reference for ballan wrasse behaviour in salmon sea cages, with similar behaviour emulated  
543 in farmed wrasse considered desirable.

544

545 Wild ballan wrasse showed a strong diurnal rhythm in behaviour in both depth and activity  
546 with a preference for shallower depths at night and increased activity during the day, a  
547 pattern similar to wild wrasse in a previous sea-cage study (Leclercq et al., 2018). This  
548 increased activity during the day suggests that active foraging and delousing behaviour occur  
549 during the day. Furthermore, although cage edges and corners were preferred locations at all  
550 times, they were used more at night than during the day, suggesting nocturnal quiescence  
551 similar to ballan wrasse in the wild (Costello, 1991; Villegas- Ríos et al., 2013). Cleaner fish  
552 hides were very seldom used by wild wrasse, as also previously reported by Leclercq et al.  
553 (2018), although corners with hides were preferred over empty corners. Underwater  
554 structures are known to be an attraction for many pelagic and demersal fish species  
555 (Pickering and Whitmarsh, 1997) suggesting that proximity to a structure may attract ballan  
556 wrasse in sea cages despite the fish rarely being resident within the hides. By stationing in  
557 open water close to the hides, these fish may be optimising feeding opportunities while  
558 remaining close enough to be able to seek shelter in or around the hide if threatened, and  
559 these areas may be where delousing occurs. Hides are considered essential for wrasse  
560 maintenance in salmon sea cages (Treasurer, 2013), and although they were used very little  
561 by the fish in this study, they do seem to have a role in providing suitable structures to attract  
562 the wrasse. As this study was conducted during the summer, it may be that hides are used  
563 more at low winter temperatures when ballan wrasse are known to enter a state of torpor  
564 (Morel et al., 2013), although hide use was also relatively low in a similar study conducted at  
565 lower temperatures in March–May (Leclercq et al., 2018). Wild wrasse spent very little time

566 in the centre of the cage where the majority of the salmon biomass can be found, and core  
567 areas of the wild wrasses' ranges (KUD<sub>50</sub>) nearly always incorporated hide corners (with  
568 some in empty corners). The attraction of cage edges and corners may also be due to the  
569 substrate-grazing habits of ballan wrasse (Deady et al., 1995; Skiftesvik et al., 2013), and  
570 while the nets were air-dried fortnightly to avoid biofouling, ballan wrasse were often  
571 observed grazing on the nets.

572

573 In contrast to the wild wrasse, the farmed wrasse in trial 1 remained deep (>15m) throughout  
574 the trial and showed no diurnal variation in depth or activity (except in activity towards the  
575 end of the trial). In addition, their home ranges were smaller than for wild wrasse and were  
576 focussed at the centre of the cage with their core areas primarily in the centre at the deepest  
577 part of the cage. This behaviour is very different to the behaviour patterns exhibited by the  
578 wild wrasse in trial 1 and is unlikely to be conducive to desirable behaviour, i.e. delousing.  
579 Considering their hatchery rearing conditions in small circular tanks with constant lighting,  
580 temperature and feeding, it is not surprising that such a dramatic change in environmental  
581 conditions, in addition to the stress of transport to the grow-out site (Leclercq et al., 2014b),  
582 resulted in the fish remaining deep at the bottom of the sea cage throughout the study  
583 possibly reflecting difficulties in coping with the change. However, it should be reiterated  
584 that the wild wrasse in trial 1 were already resident in the sea cage for several months prior to  
585 tagging and were already acclimatised to the farm conditions at the start of the trial.

586

587 A further effect of tank rearing can be seen in the strong orientation of the farmed wrasse to  
588 tidal currents in the sea cage. While the wild wrasse in trial 1 showed a relatively even  
589 distribution of headings, the farmed wrasse showed a strong orientation to the predominant  
590 tidal currents at the sea-cage site. This is likely due to the farmed wrasse becoming



591 acclimatised to the uniform circular current present in the rearing tanks, and they commonly  
592 orientate towards this current. In comparison, wild wrasse are accustomed to the swirling  
593 currents and eddies of natural rocky reefs. The domestication of fish is a strong selection  
594 pressure and results in the development of specific behavioural syndromes (Sih et al., 2004;  
595 Huntingford and Adam, 2005) as seen in the farmed wrasse in this study, which may not be  
596 desirable traits for ballan wrasse used as cleaner fish.

597

598 While the comparison of different groups of fish allows differing trends in behaviour to be  
599 identified, a significant benefit of PAT is the ability to quantify the behaviour of individual  
600 fish. Despite fish in the same group being treated the same, there was large variation amongst  
601 individual fish behaviours, and consistent individual differences in behaviour result in  
602 various behavioural phenotypes, or personalities, within a population (Huntingford, 2004;  
603 Nilsson et al., 2014). In wild wrasse in trial 1, some fish remained deep (>15m), whereas  
604 others preferred shallower depths (<10m). Furthermore, some fish showed no diurnal  
605 variation in depth, where others preferred shallower depths at night than during the day.

606 While there was less variation between the activities of individual fish, all but one wild  
607 wrasse had significantly higher activity during the day than at night. This suggests that the  
608 wild wrasse were nocturnally quiescent with resting activity rates at night generally <0.3  
609 BL/s and actively foraging during the day with activity rates of 3–6 BL/s although it remains  
610 to be proven whether increased swimming activity translates into increased delousing  
611 activity.

612

#### 613 4.3 Effect of acclimatisation to sea-cage conditions

614 Due to the consistently deep mean depths, low activity and lack of diurnal behaviour patterns  
615 observed in the farmed wrasse studied in trial 1, the acclimatisation trials aimed to explore

616 methods of improving the behaviour of farmed ballan wrasse following deployment. The  
617 acclimatisation protocol was implemented as a two-step process in two separate trials to  
618 determine the relative importance of acclimatisation in the hatchery and acclimatisation in the  
619 sea cage.

620

621 Trial 1 and trial 2 were conducted in different years (2015 and 2016) with different stocks of  
622 farmed wrasse, albeit from the same hatchery, so it is difficult to draw conclusions regarding  
623 differences in behaviour between the two trials. Ballan wrasse culture is a new and rapidly  
624 evolving industry (Brooker et al., 2018b), and culture techniques are likely to have changed  
625 between the two cohorts of fish being produced. Indeed, the fish used in the first trial in 2015  
626 were some of the first farmed ballan wrasse to be deployed in salmon cages in Scotland. This  
627 may have been a contributing factor explaining the higher mortalities in the farmed wrasse in  
628 trial 1 and differences in behaviour between these fish and the non-acclimatised farmed  
629 wrasse in trials 2a and 2b. Furthermore, although study 1 and study 2a were conducted at  
630 approximately the same time of year with similar water temperatures, salinity was lower in  
631 study 1, especially near the surface, due to high rainfall and freshwater runoff during the  
632 study. This may partially explain differences in the depth of the fish between trials 1 and 2a.  
633 Territorial competition between the wild and farmed wrasse present in the same cage also  
634 cannot be discounted.

635

636 The hatchery acclimatisation using artificial kelp hides, natural photoperiod and  
637 supplementary feed blocks (trial 2a), had no apparent significant impact on depth and just one  
638 fish developed diurnal rhythms in activity. Conversely, hatchery acclimatisation followed by  
639 acclimatisation in the sea cage where fish were retained in keep nets within the sea cages  
640 with artificial kelp hides and supplementary feed blocks (trial 2b) had a significant impact on

641 behaviour, especially for depth and activity and the development of diurnal activity patterns.  
642 However, study 2b was conducted later in the summer when water temperatures were higher,  
643 and this may partially account for the higher rates of activity and shallower swimming depths  
644 in this study. There was a broad range in the mean activities of individual hatchery-and-cage-  
645 acclimatised wrasse, and not all fish developed diurnal activity patterns. It seems that this  
646 diurnal pattern in activity is an indicator of a positive response to acclimatisation and the  
647 development of daily rhythms in behaviour, similar to those of wild wrasse in trial 1. The  
648 different responses of individual fish may be due to differences in their behavioural plasticity  
649 (Dingemanse and Wolf, 2013) with some fish able to adapt to the sea-cage environment more  
650 effectively than others. Villegas-Ríos et al. (2018) found that the response of wild cod  
651 (*Gadus morhua*) to environmental fluctuations can be partially explained by their different  
652 personalities on a reactive-proactive axis, and this may contribute to the different responses  
653 of acclimatised fish in this study. However, genetics and environmental fluctuations can only  
654 partially explain differences in individual fish behaviour, and even in consistent and identical  
655 rearing conditions, as in farmed wrasse in this study, behavioural individuality has been  
656 demonstrated in clonal fish, and individuality may be an inevitable outcome of early  
657 development (Bierbach et al., 2017). Nonetheless, while a range of behavioural phenotypes  
658 was observed in the hatchery-and-cage-acclimatised wrasse, the acclimatisation process  
659 appears to have shifted the overall population behaviour structure towards that of wild  
660 wrasse.

661

662 The positive effect of hatchery-and-sea-cage acclimatisation is further evidenced by cage  
663 locations. Cage edges and corners were preferred by the hatchery-and-cage-acclimatised  
664 wrasse during the daytime and night time, similar to the preferences of wild wrasse seen in  
665 trial 1. Hatchery-and-cage acclimatisation also had a positive effect on reducing orientation to

666 prevailing currents, which appears to be a result of being reared in tanks with consistent  
667 directional currents. While trial 2b non-acclimatised wrasse were strongly oriented to the  
668 prevailing ESE-WNW tidal currents at the site, the headings of hatchery-and-cage-  
669 acclimatised wrasse were more evenly distributed, as also seen in the wild wrasse. Although  
670 the sizes and cage coverage of the home ranges and core areas were similar for both groups  
671 of fish in trial 2b, the estimation of cumulative home ranges highlights a clear difference  
672 between acclimatised and non-acclimatised wrasse. The establishment of home ranges was  
673 rapid in hatchery-and-cage-acclimatised wrasse (mean  $8.1 \pm 4.3$  d), while non-acclimatised  
674 wrasse gradually expanded their home ranges over a longer period (mean  $10.9 \pm 5.02$  d),  
675 which provides further evidence that the hatchery-and-cage acclimatisation promoted the  
676 rapid acclimatisation of the fish to the sea-cage environment.

677

## 678 **5 CONCLUSIONS**

679 Ballan wrasse are proven to be effective at delousing salmon infected with sea lice in  
680 commercial sea cages, although the origin of the fish, wild or farmed, appears to impact on  
681 delousing efficiency. In this study, PAT was used effectively to visualise the behaviour of  
682 individual cleaner fish in commercial salmon sea cages and compare wild and farmed wrasse  
683 and investigate the effect of acclimatisation. Clear differences were found between the  
684 behaviour of wild and farmed ballan wrasse. Wild wrasse frequented shallower depths, were  
685 more active during the day and covered more of the cage area. A combination of hatchery-  
686 and-cage acclimatisation to sea-cage conditions had a positive effect on farmed wrasse, with  
687 these fish exhibiting behaviours similar to those of wild origin and more rapidly acclimatising  
688 to the sea-cage environment. The development of diurnal rhythms in behaviour, particularly  
689 in activity, appears to be a key indicator of a positive response to acclimatisation in farmed  
690 wrasse. While most hatchery-and-cage-acclimatised fish developed diurnal rhythms in

691 activity in this study, some did not, and it may be that the response of individual fish to  
692 acclimatisation is a function of time, with some fish requiring longer acclimatisation periods  
693 than others. Extending the acclimatisation period may further shift their overall behaviour  
694 towards that of wild wrasse, although further investigation is required. Nonetheless, a  
695 combination of hatchery-and-cage acclimatisation is recommended prior to deployment to  
696 improve farmed ballan wrasse delousing efficacy.

697

698

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707

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800

**Table 1.** Summary of the three acoustic tagging trials.

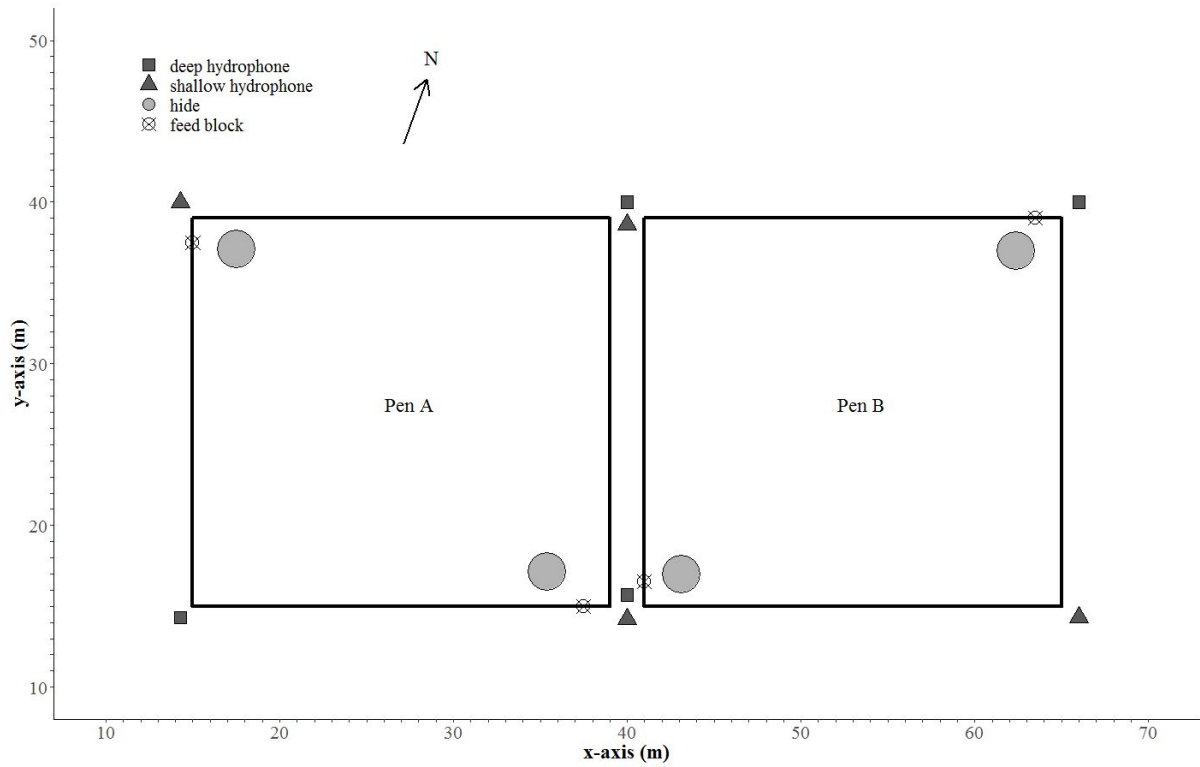
	<b>Trial 1</b>	<b>Trial 2a</b>	<b>Trial 2b</b>
Comparison	Wild vs. non-acclimatised farmed ballan wrasse	Hatchery-acclimatised vs. non-acclimatised farmed ballan wrasse	Hatchery-and-cage-acclimatised vs. non- acclimatised farmed ballan wrasse
Hatchery acclimatisation	–	11 <sup>th</sup> May – 19 <sup>th</sup> June 2016	5 <sup>th</sup> August – 30 <sup>th</sup> August 2016
Sea-cage acclimatisation	–	–	30 <sup>th</sup> August – 12 <sup>th</sup> September 2016
Transport to sea cage	Wild: October 2014, Farmed: 31 <sup>st</sup> May 2015	11 <sup>th</sup> June 2016	Acclimatised: 30 <sup>th</sup> August 2016, non- acclimatised: 11 <sup>th</sup> September 2016
Tagging surgery	2 <sup>nd</sup> June 2015	13 <sup>th</sup> June 2016	12 <sup>th</sup> September 2016
No. tagged wrasse	18 per group	20 per group	17 per group
Weight at surgery	Wild: 66.27 ± 12.21 g Farmed: 43.57 ± 6.49 g	Acclimatised: 45.08 ± 5.39 g, non- acclimatised: 46.38 ± 4.92 g	Acclimatised: 50.43 ± 6.23 g, non- acclimatised: 63.70 ± 11.73 g
Length at surgery (TL)	Wild: 16.83 ± 1.01 cm Farmed: 13.97 ± 0.60 cm	Acclimatised: 13.38 ± 0.57 cm, non- acclimatised: 13.59 ± 0.42 cm	Acclimatised: 14.35 ± 0.67 cm, non- acclimatised: 14.79 ± 0.73 cm
Deployment date	5 <sup>th</sup> June 2015	15 <sup>th</sup> June 2016	14 <sup>th</sup> September 2016
Data acquisition	124 days	60 days	77 days

**Table 2.** Summary of water quality parameters at 4m depth during the three acoustic trials recorded at 30 min intervals and averaged over 12 h. (mean  $\pm$  standard deviation, range in parentheses).

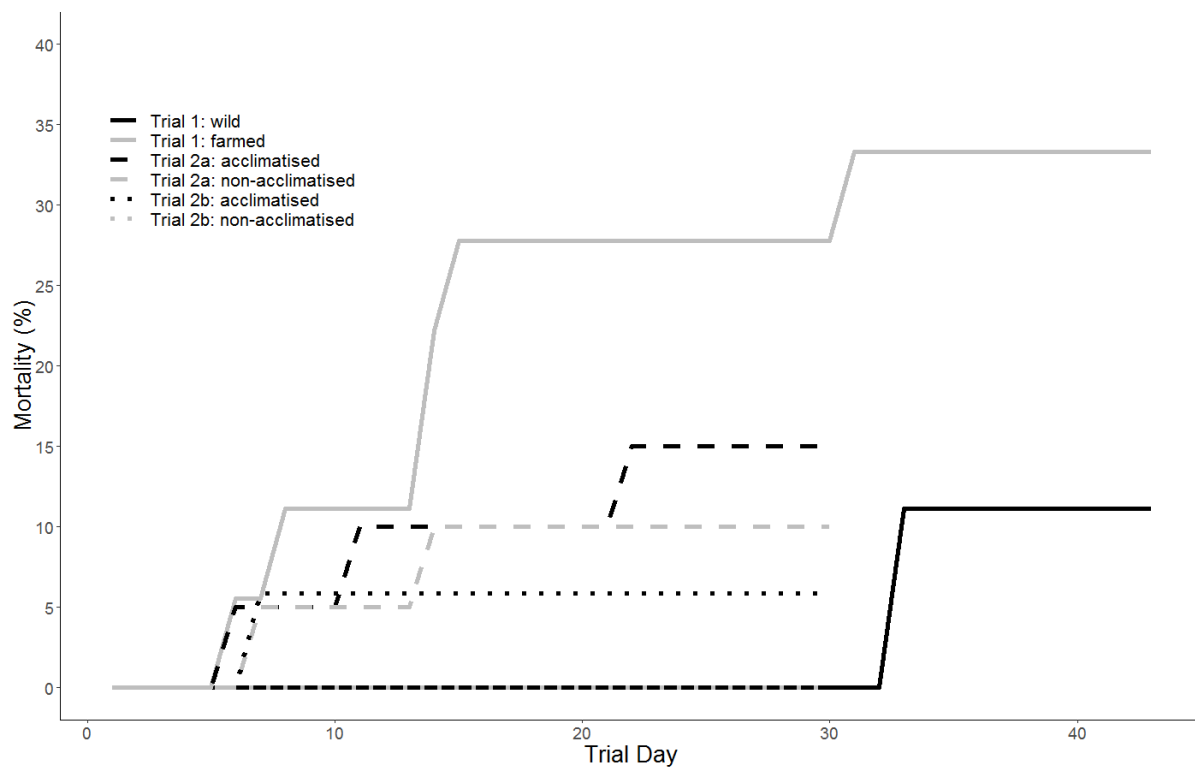
	<b>Trial 1</b>	<b>Trial 2a</b>	<b>Trial 2b</b>
Temperature (°C)	11.59 $\pm$ 0.97 (9.01– 13.20)	11.68 $\pm$ 0.42 (10.59– 12.82)	13.66 $\pm$ 0.29 (12.92– 14.18)
Salinity (PSU)	18.83 $\pm$ 6.04 (9.53– 26.92)	32.49 $\pm$ 0.57 (31.16– 33.54)	29.38 $\pm$ 4.37 (21.29– 36.88)
Dissolved oxygen (mg/L)	9.70 $\pm$ 1.60 (4.64– 13.11)	10.36 $\pm$ 0.42 (9.40– 11.48)	7.11 $\pm$ 0.67 (4.67– 8.85)

**Table 3.** Core areas (CA) and home ranges (HR) of individual fish in each trial. All values are m<sup>2</sup>.

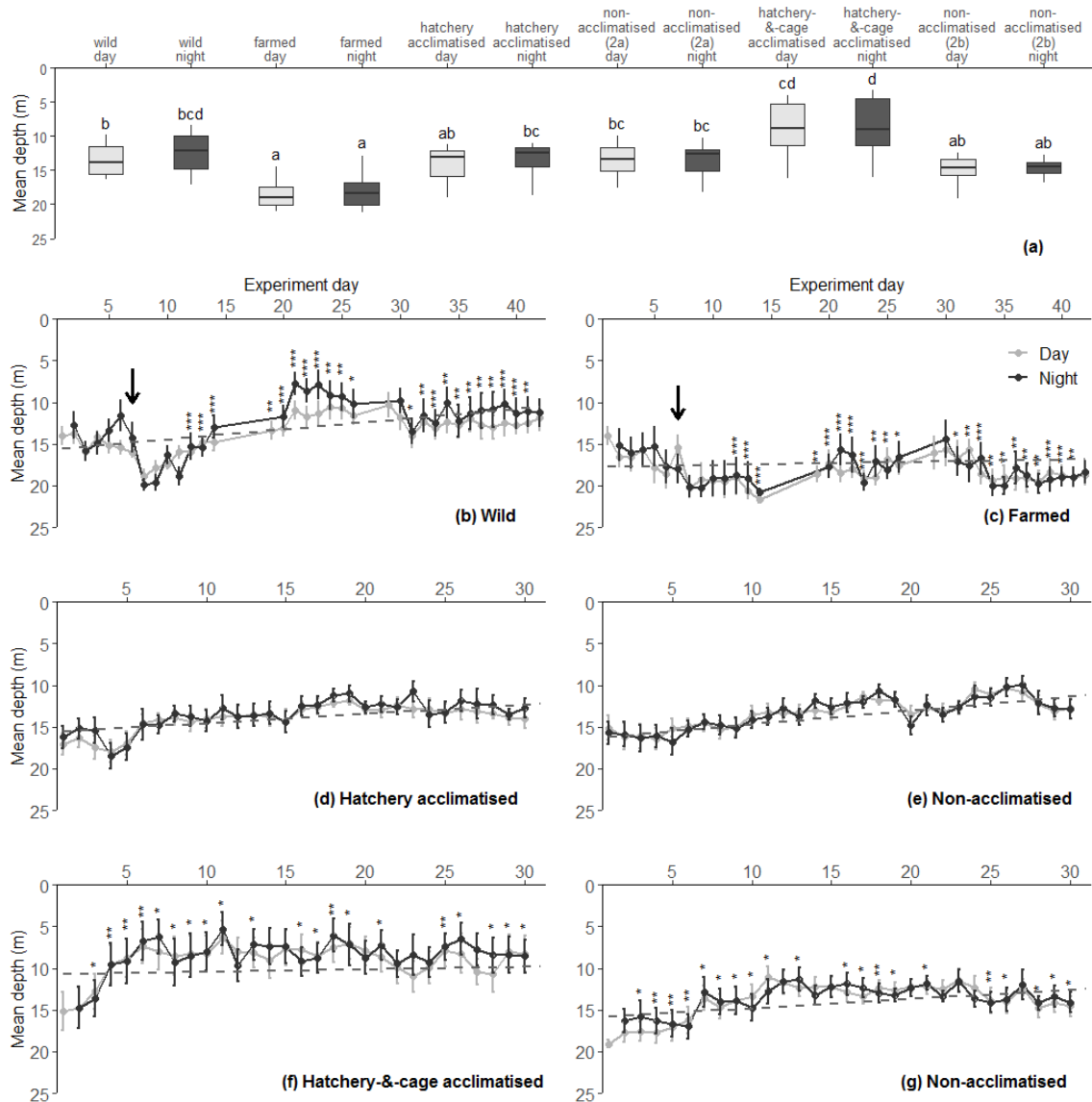
Fish ID	Trial 1				Trial 2b			
	Wild CA	Wild HR	Farmed CA	Farmed HR	Acclimatised CA	Acclimatised HR	Non-acclimatised CA	Non-acclimatised HR
1	95	680	23	237	95	564	40	425
2	148	733	70	364	118	599	28	194
3	123	664	14	194	14	142	65	440
4	37	407	24	339	82	611	75	562
5	121	566	108	648	75	572	62	563
6	83	627	10	185	76	474	80	576
7	26	546	85	457	68	553	24	303
8	113	583	43	355	117	676	35	347
9	138	709	29	314	84	481	25	412
10	85	642	22	369	55	472	11	138
11	140	674	-	-	-	-	43	398
12	98	589	-	-	-	-	55	421
13	101	637	-	-	-	-	65	439
14	63	580	-	-	-	-	40	449
Mean ±	97.8 ±	616.8 ±	42.9 ±	346.0 ±	78.3 ±	514.3 ±	46.1 ±	404.6 ±
SD	36.8	110.1	33.4	136.1	30.2	146.6	20.9	128.2



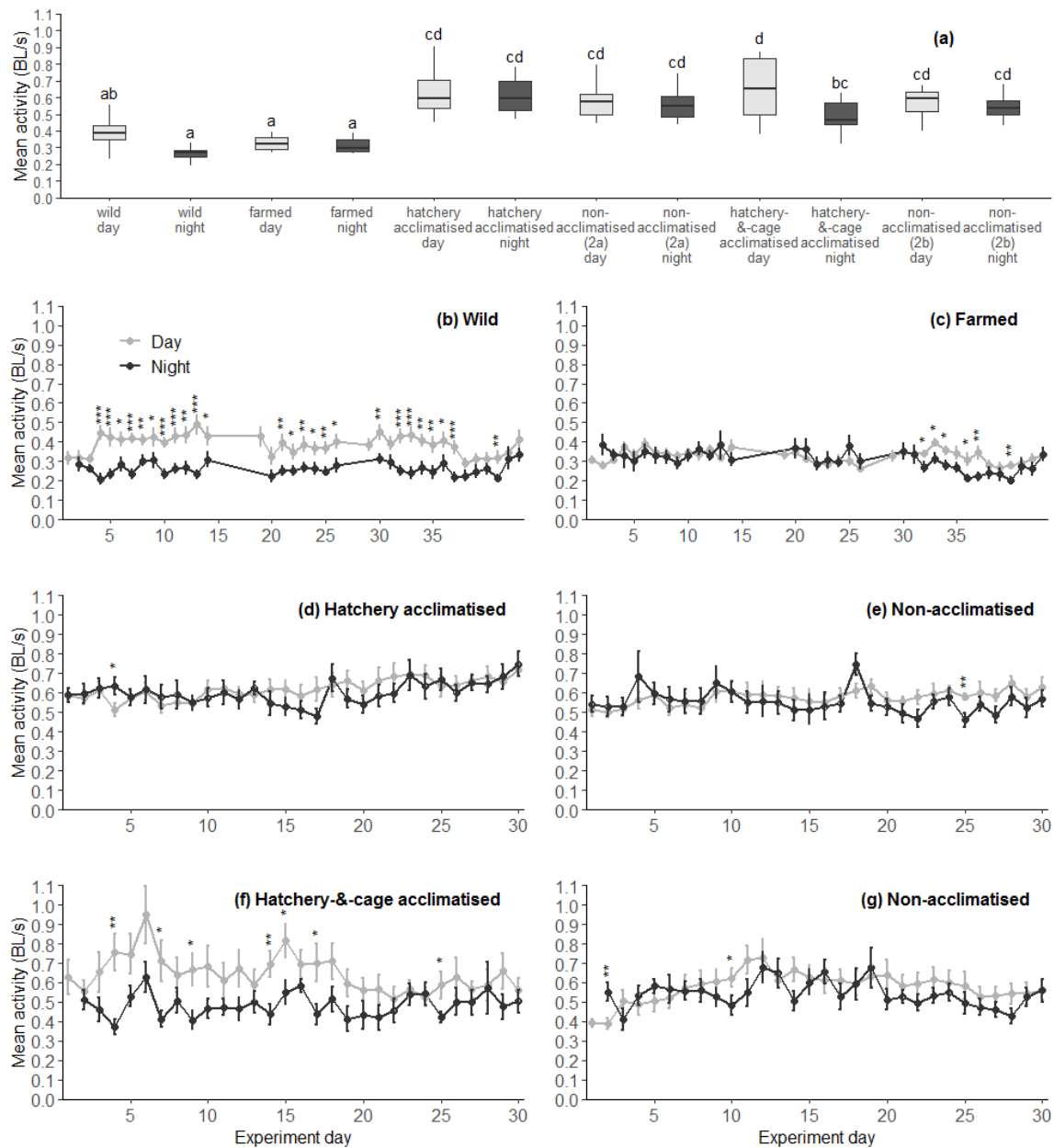
**Figure 1.** Plan-view schematic of  $24 \times 24$  m square cages in the local Cartesian coordinate system showing the positioning of the cleaner-fish hide areas (2 m diameter grey circles, mean location during study), the hydrophone array deployed across two horizontal planes at 1 m (shallow) and 20 m (deep) depth and wrasse feed blocks deployed weekly adjacent to the hides.



**Figure 2.** Ballan wrasse mortality in all trials and experimental groups during the periods used for analysis.



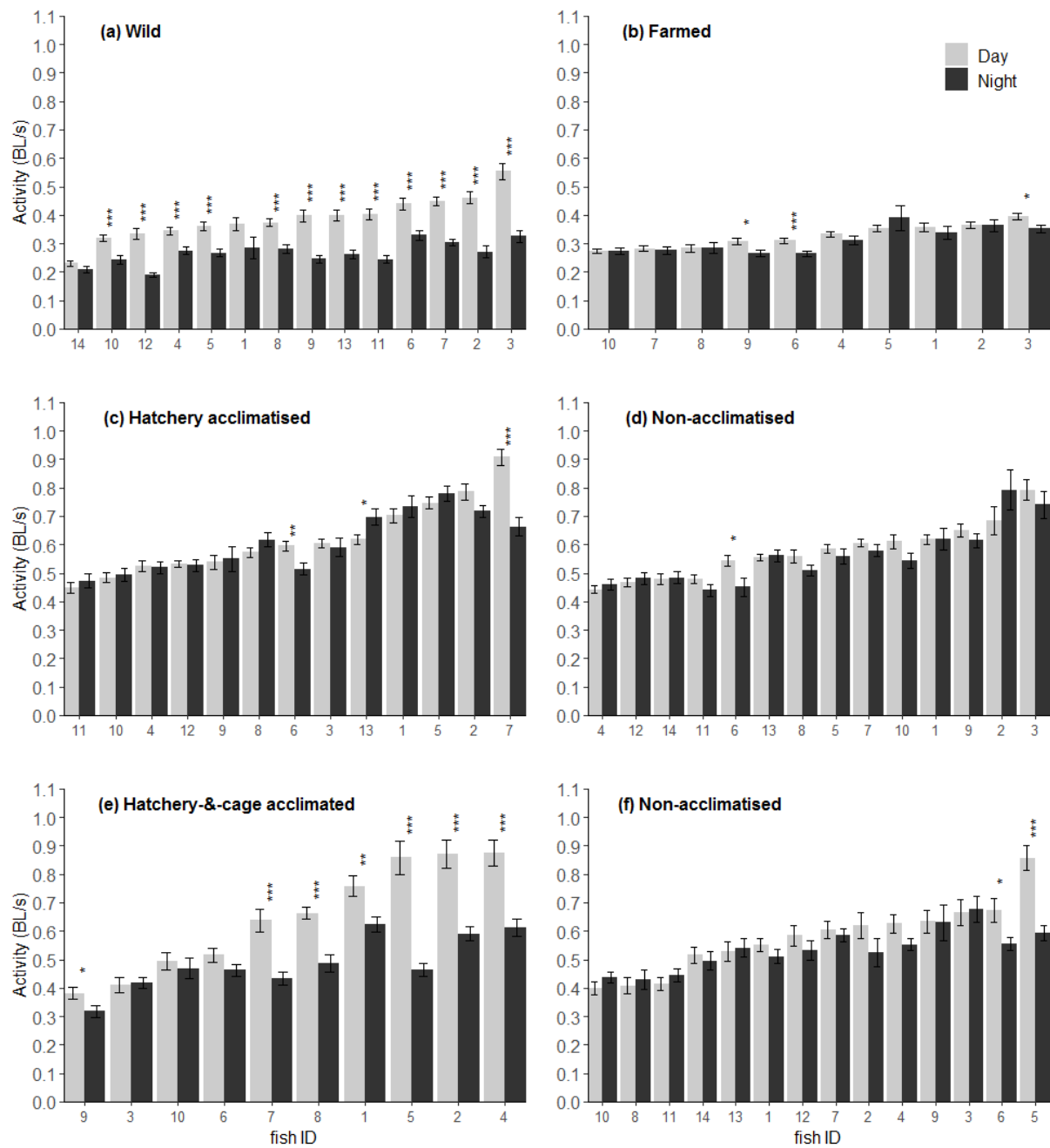
**Figure 3.** (a) Boxplot of group depth for each trial (letters represent significance with different letters denoting significant differences from other groups,  $P < 0.05$ ). Comparison of daytime and night time mean daily depth for (b) trial 1 wild wrasse, (c) trial 1 farmed wrasse, (d) trial 2a hatchery-acclimatised wrasse, (e) trial 2a non-acclimatised wrasse, (f) trial 2b hatchery-and-cage-acclimatised wrasse and (g) trial 2b non-acclimatised wrasse (mean  $\pm$  SEM,  $n = 10\text{--}14$  fish.). Asterisks show significant differences between daily mean depths of corresponding treatment groups (\* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ ). Dotted lines are fitted linear regressions of daily mean activity. Gaps between points in (b) and (c) are due to temporary system failures. Arrows indicate day of bath treatment.



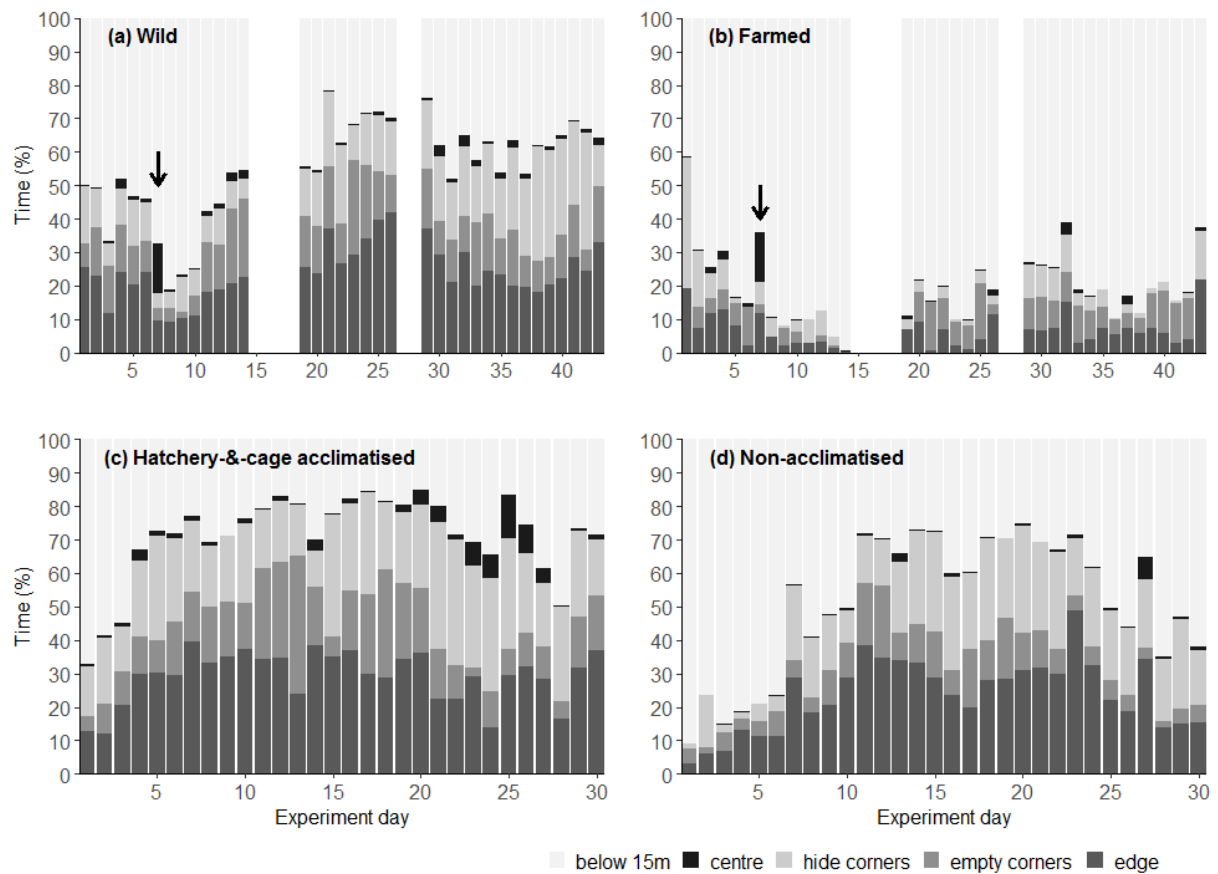
**Figure 4.** (a) Boxplot of group activity for each trial (letters represent significance groups with different letters denoting significant differences from other groups,  $P < 0.05$ ).

Comparison of daytime and night time mean daily activity for (b) trial 1 wild wrasse, (c) trial 1 farmed wrasse, (d) trial 2a hatchery-acclimatised wrasse, (e) trial 2a non-acclimatised wrasse, (f) trial 2b hatchery-and-cage-acclimatised wrasse and (g) trial 2b non-acclimatised wrasse (mean  $\pm$  SEM,  $n = 10\text{--}14$  fish. Asterisks show significant differences between daytime and night time activity (\* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ ). Gaps between points in (b) and (c) are due to temporary network failures.

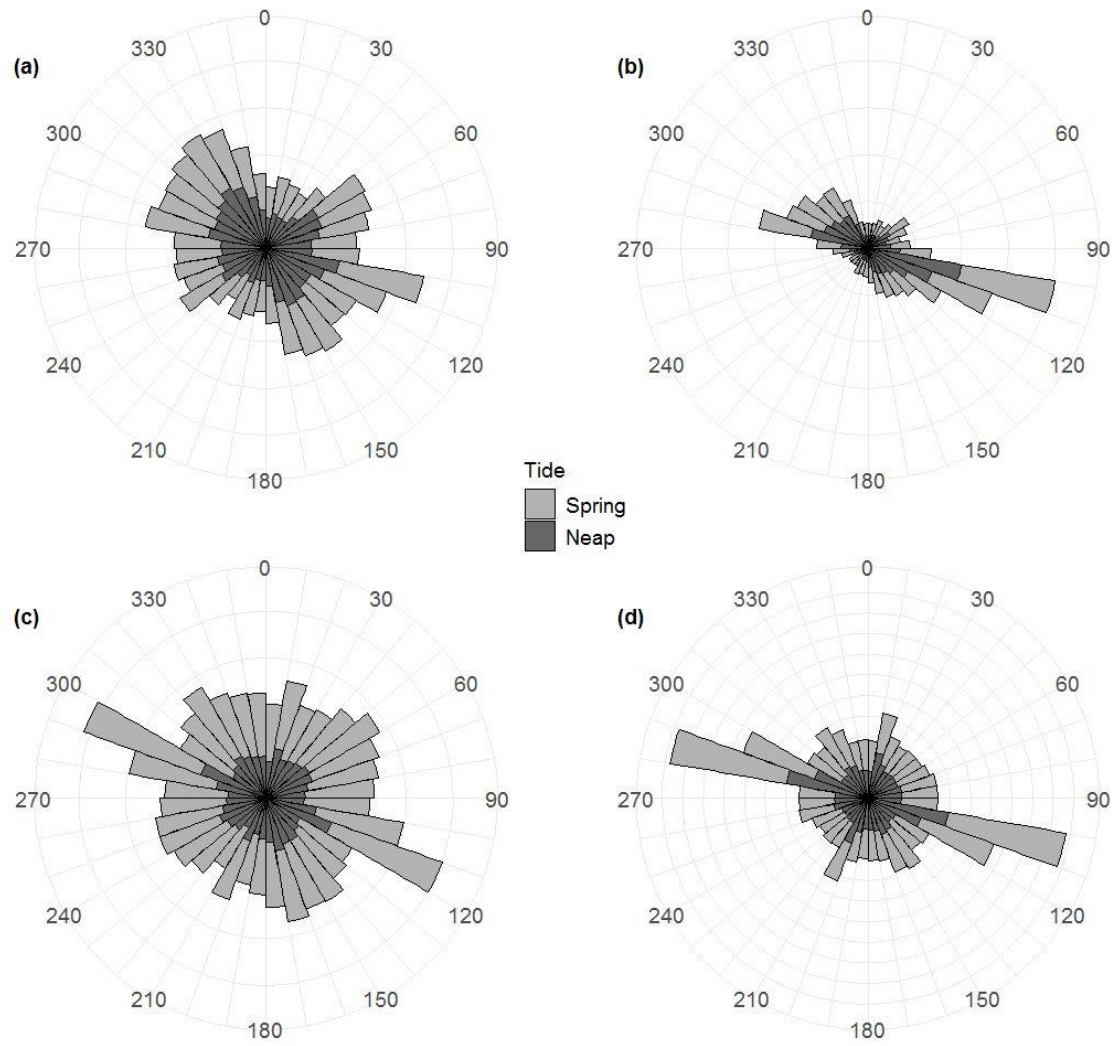




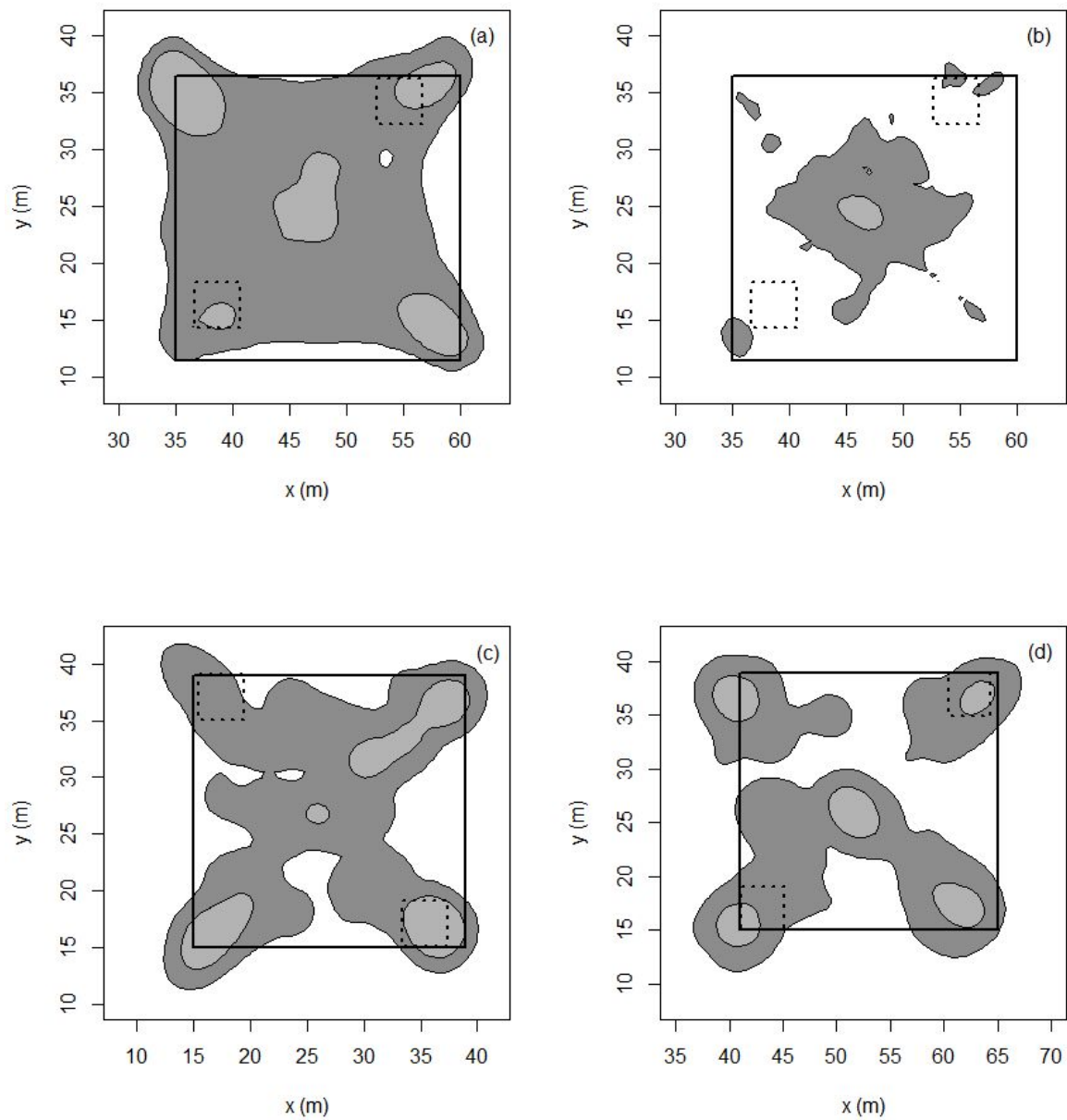
**Figure 5.** Comparison of daytime and night time mean activity of individual fish for (a) trial 1 wild wrasse, (b) trial 1 farmed wrasse, (c) trial 2a hatchery-acclimatised wrasse, (d) trial 2a non-acclimatised wrasse, (e) trial 2b hatchery-and-cage-acclimatised wrasse, (f) trial 2b non-acclimatised wrasse (mean  $\pm$  SEM, n = 30–43 days). Asterisks show significant differences between daytime and night time activity (\* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ ).



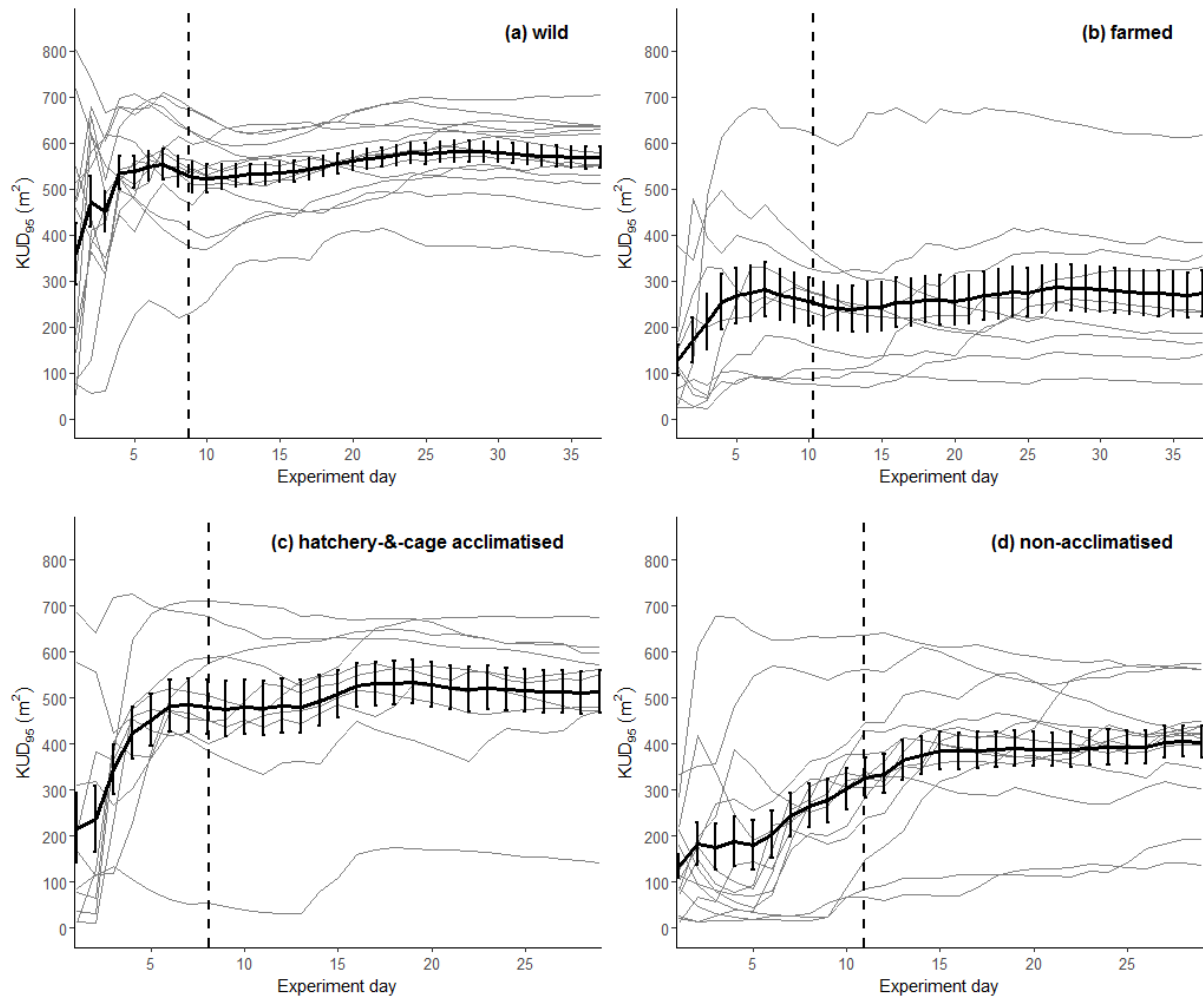
**Figure 6.** Proportion of time spent in different cage locations during the daytime for (a) trial 1 wild wrasse, (b) trial 1 farmed wrasse, (c) trial 2b hatchery-and-cage-acclimatised wrasse, (d) trial 2b non-acclimatised wrasse. Gaps in (a) and (b) are due to temporary system failures. Arrows indicate day of bath treatment.



**Figure 7.** Polar distribution of ballan wrasse headings during spring and neap tides where swimming speed > 1 BL/s; (a) trial 1 wild wrasse, (b) trial 1 farmed wrasse, (c) trial 2b acclimatised wrasse and (d) trial 2b non-acclimatised wrasse (y-axis gridlines = 2,500 observations).



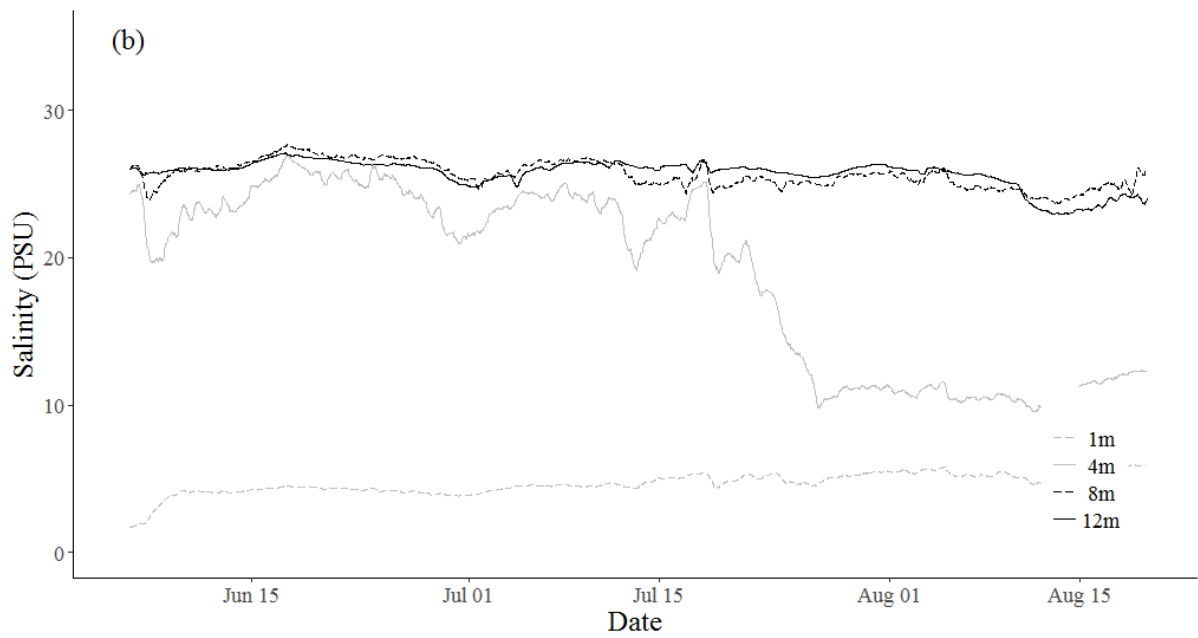
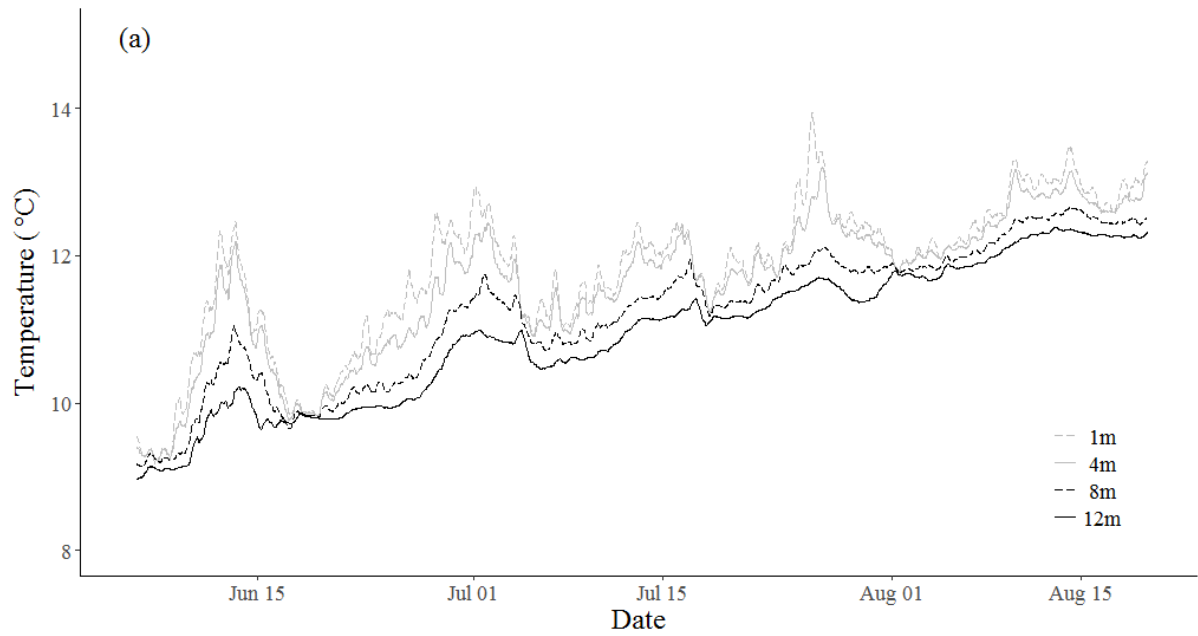
**Figure 8.** Examples of kernel utilisation distributions (home ranges) of individual fish for (a) wild wrasse #11, (b) farmed wrasse #3, (c) hatchery-and-cage-acclimatised wrasse #9 and (d) non-acclimatised wrasse #3. Light grey = core area ( $KUD_{50}$ ), dark grey = home range ( $KUD_{95}$ ), solid line = cage boundary and dotted lines = approximate location of hides.

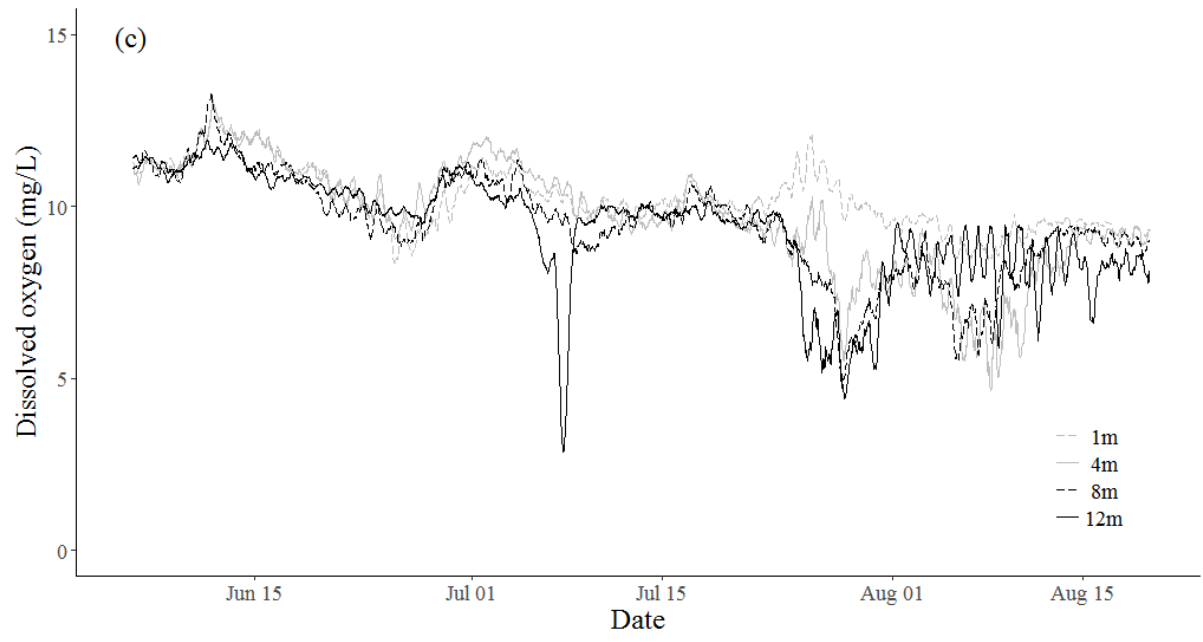


**Figure 9.** Cumulative 95% kernel utilisation distributions ( $KUD_{95}$ ) for (a) wild wrasse, (b) farmed wrasse, (c) hatchery-and-cage-acclimatised wrasse and (d) non-acclimatised wrasse. Grey lines = cumulative  $KUD_{95}$  for individual fish, black lines = mean cumulative  $KUD_{95} \pm$  SEM, dotted lines = mean asymptote (time taken to establish home range).

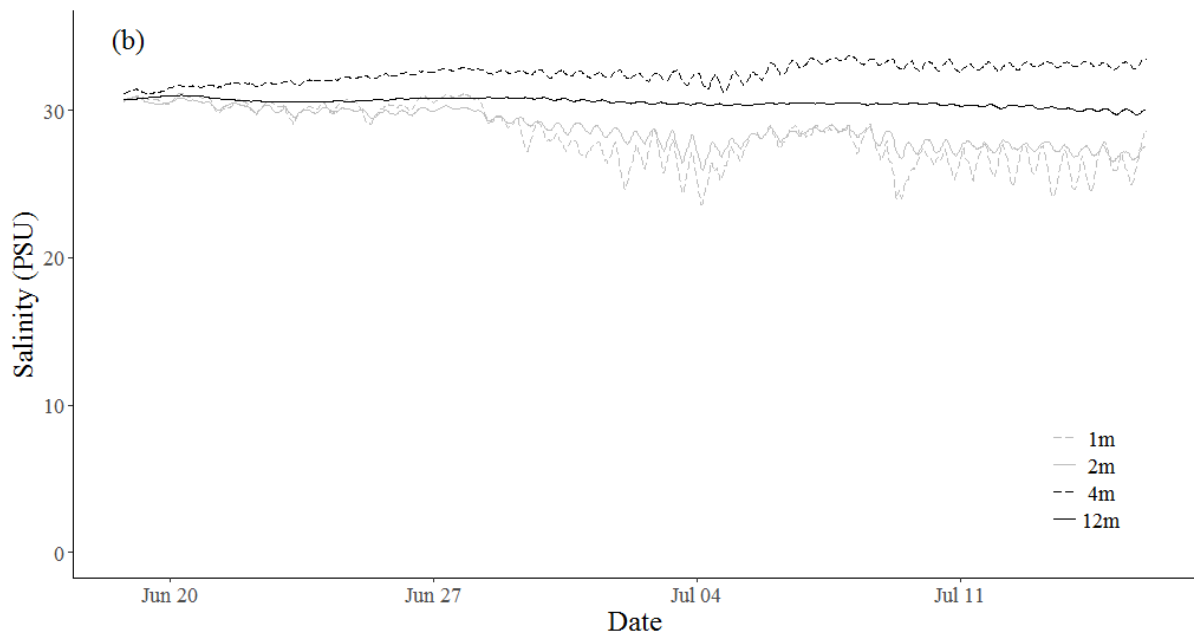
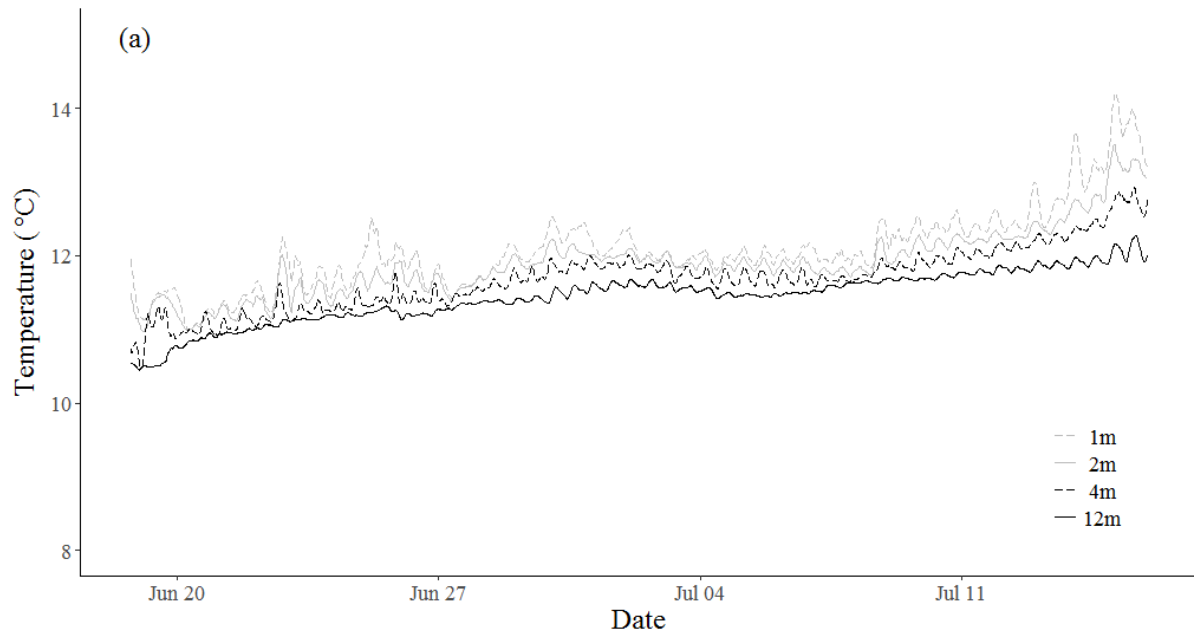
## Supplementary data

**S1.** (a) temperature, (b) salinity and (c) dissolved oxygen during trial 1 measured at 1, 4, 8 and 12m depth and 30 min intervals over the study period and averaged over 12 h.

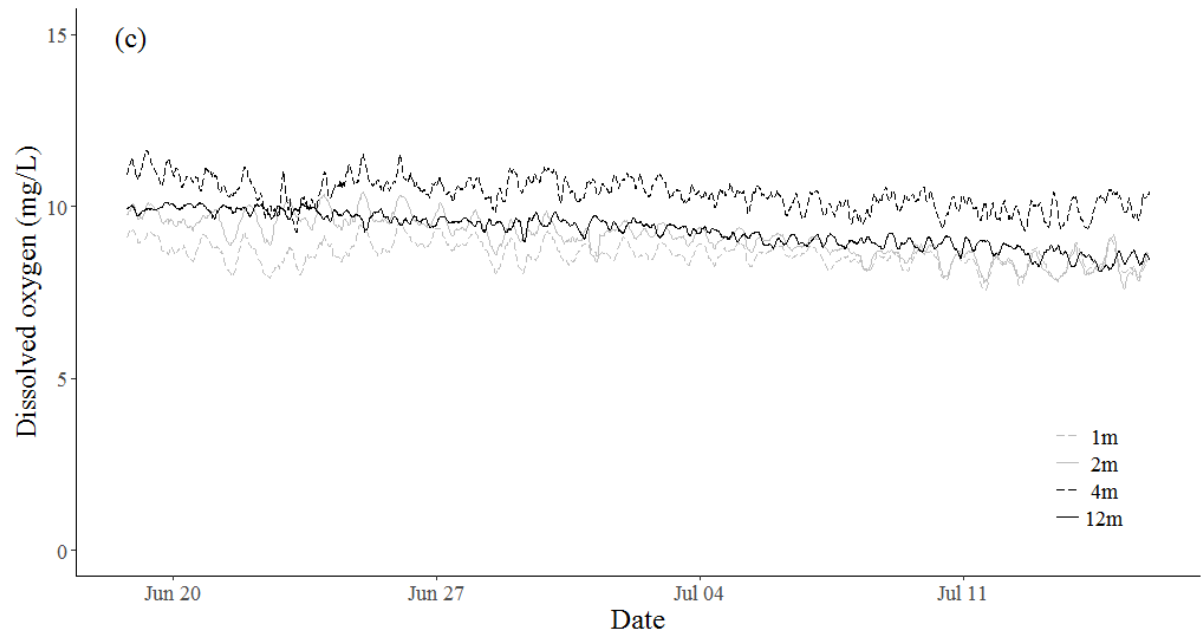




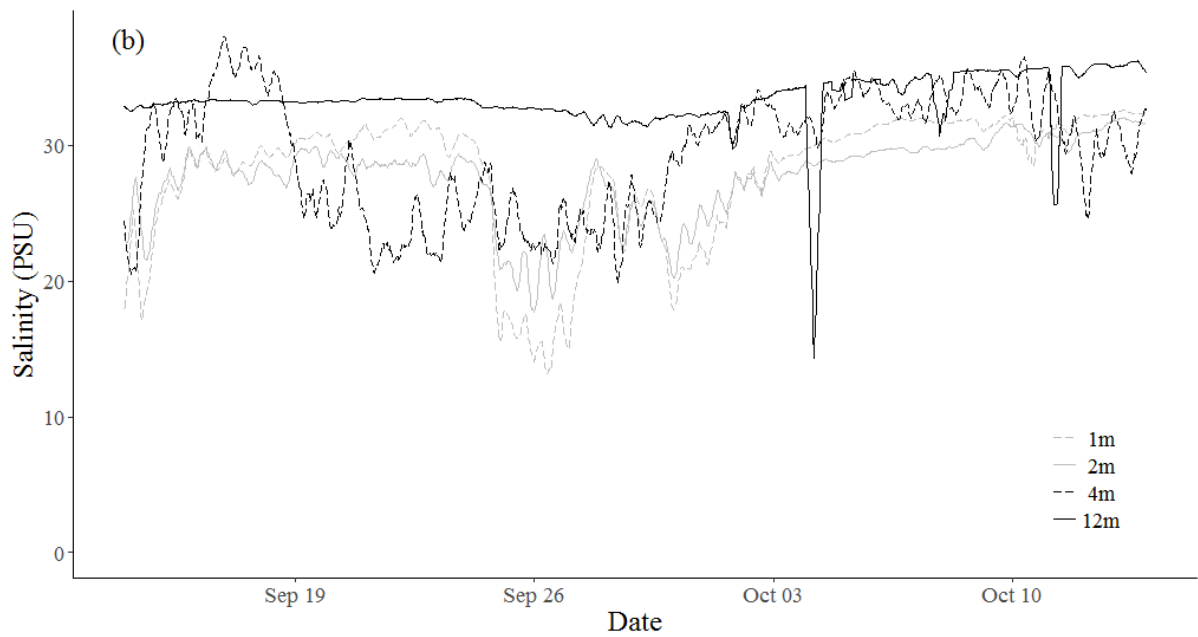
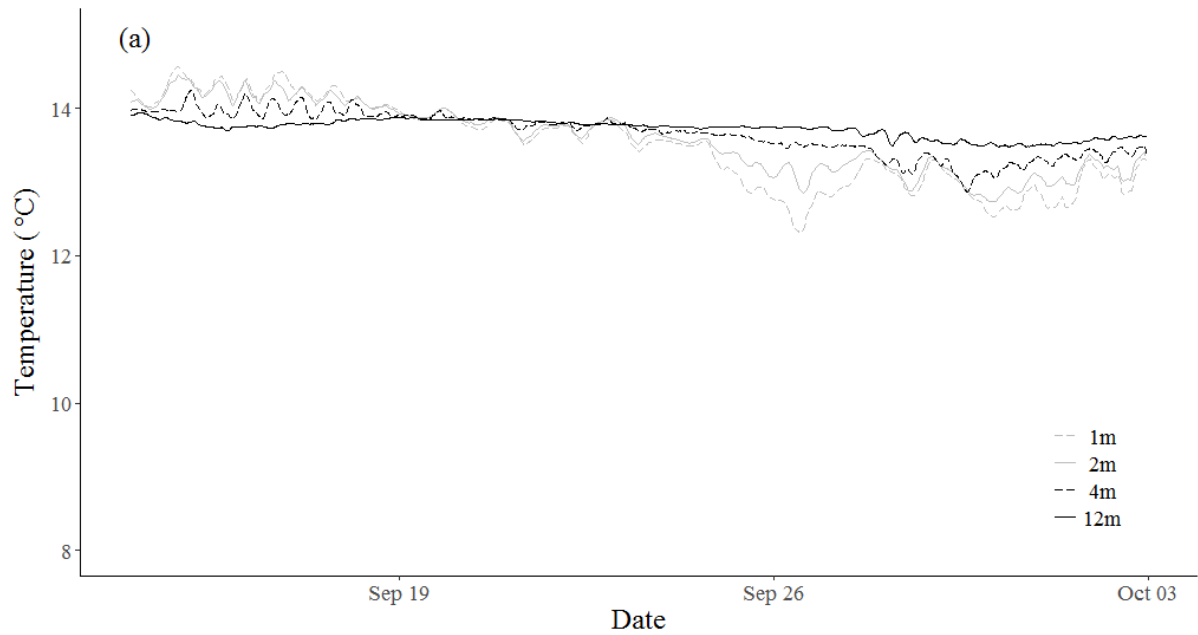
**S2.** (a) temperature, (b) salinity and (c) dissolved oxygen during trial 2a measured at 1, 2, 4 and 12m depth and 30 min intervals over the study period and averaged over 12 h.

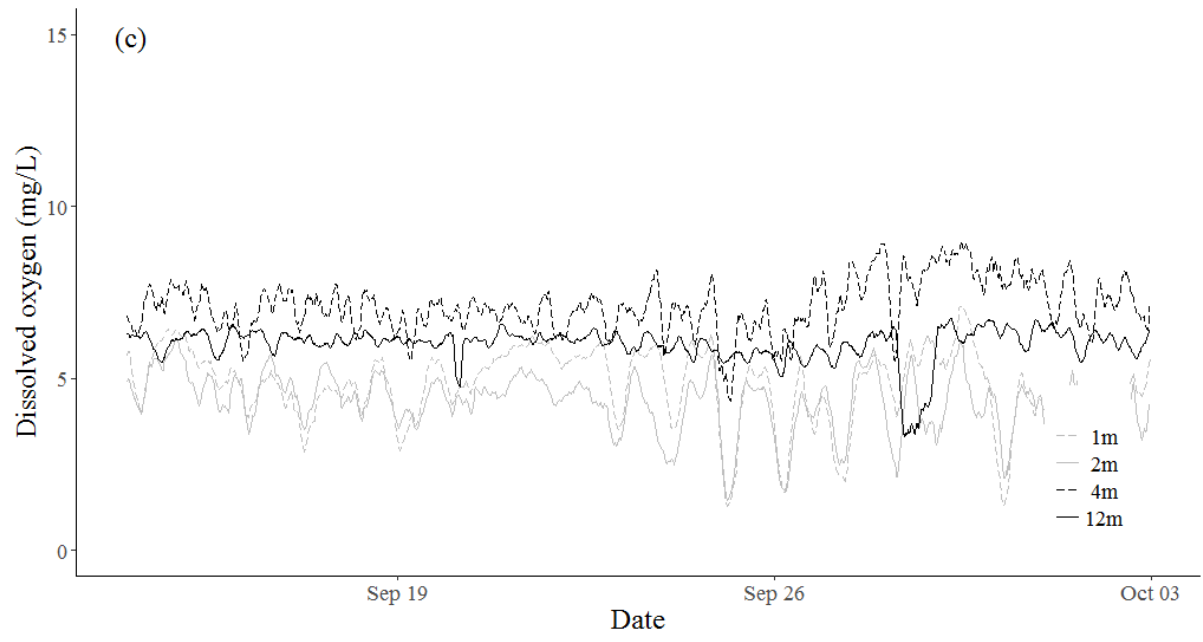




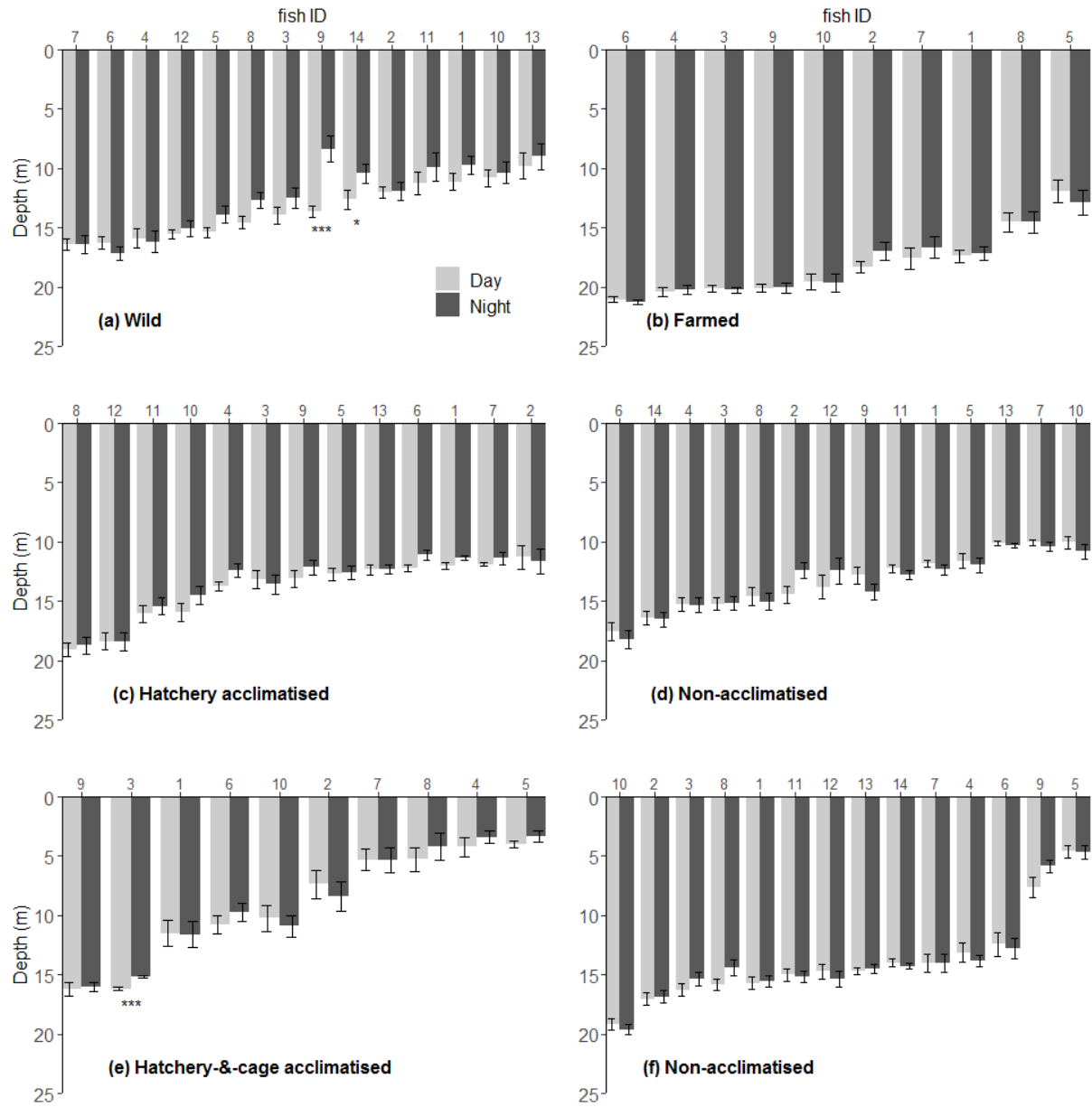


**S3.** (a) temperature, (b) salinity and (c) dissolved oxygen during trial 2b measured at 1, 2, 4 and 12m depth and 30 min intervals over the study period and averaged over 12 h.





**S4.** Comparison of daytime and night time mean depth of individual fish for (a) trial 1 wild wrasse, (b) trial 1 farmed wrasse, (c) trial 2a hatchery-acclimatised wrasse, (d) trial 2a non-acclimatised wrasse, (e) trial 2b hatchery-and-cage-acclimatised wrasse, (f) trial 2b non-acclimatised wrasse (mean  $\pm$  SEM, n = 30–43 days). Asterisks show significant differences between daytime and night time depths (\* = P <0.05, \*\*\* = P <0.001).



**S5.** Proportion of daily time spent in different cage locations during the night time for (a) trial 1 wild wrasse, (b) trial 1 farmed wrasse, (c) trial 2b hatchery-and-cage-acclimatised wrasse (d) trial 2b non-acclimatised wrasse. Gaps in (a) and (b) are due to temporary system failures. Arrows indicate the night following the bath treatment.

