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Development of a water-stable agar-based diet for the supplementary feeding of cleaner fish ballan wrasse (*Labrus bergylta*) deployed within commercial Atlantic salmon (*Salmon salar*) net-pens.

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Abstract

The aim of this project was to develop a water-stable and palatable diet for the supplementary feeding of wrasse deployed in salmon sea-pens using a gelling agent mixed with a manufactured dry-feed component. Three binders (gelatine from cold water fish skin, beef gelatin and agar-agar) were compared for water-gel strength over a range of concentrations. Gel formed using agar was found to be significantly stronger than the other binders tested. An experimental aqua-feed made using a grinded, dry ingredient mix binded with 20 g/L agar solution at 1/1.6 (w/v) ratio and offered as blocks within individual feeders was water-stable for 7 days when deployed fresh or following a week of preservation at -20 °C. Farmed ballan wrasse in tanks fed on the agar-based diet within 2 days of deployment. Wild wrasse stocked in salmon sea-pens at low density (1.2 to 2.1 %), up to 4 weeks prior the start of the trial and not previously fed a manufactured diet first ingested the agar feed within 2 weeks and total feed intake significantly increased afterwards. Feed intake was significantly higher from feeders placed within a small feeding shelter made of artificial kelp than within the large wrasse shelter. No nutrient leaching after water immersion and no alterations in the fatty acid profile after preparation of the experimental feed was found. A manufactured grinded ingredient mix binded with 20 g/L agar solution at a 1/1.6 (w/v) ratio and offered within static feeders is proposed as the basis of a novel supplementary feeding methodology for cleaner fish wrasse deployed in salmon sea-pens. This methodology has the potential to facilitate wrasse feeding and to allow the monitoring of feed intake to safeguard the health, welfare and delousing activity of the biological stock over the salmon rearing cycle.

1. Introduction

Cleaner fish, first tested in late 1980s (Rae, 2002), are increasingly acknowledged as an effective and sustainable biological treatment against sea-lice (Skiftesvik et al., 2013; Leclercq et al., 2014) with clear benefits over the use of chemotherapeutants. Cleaner fish are typically deployed preventively within commercial salmon net-pens and seldom exposed to high sea-lice density due to mandatory or voluntary treatment at pre-defined trigger levels. Net bio-fouling, known to act as an alternative feed source for the cleaner fish stock (Deady et al., 1995) is typically kept minimal to maintain biological delousing and the performance of the rearing system. Under such conditions, a decrease in the condition factor and body-mass of ballan wrasse (*Labrus bergylta*) was evident within 6 weeks of deployment despite a relatively high initial lice density (9 lice/salmon) and documented delousing activity (Skiftesvik et al., 2013). In a comparative tank trial, farmed ballan wrasse stocked at 5 wrasse /100 salmon and exposed to as much as 12 motile lice per salmon consumed over 75% of available sea-lice, i.e. ~ 180 sea-lice per wrasse, within 24 h (Leclercq et al., 2014). Such high lice consumption levels were not negatively affected by supplementary feeding on fresh crushed blue-mussels and did not satisfy satiation based on the functional predatory response of wrasse to sea-lice density. It is becoming increasingly evident that the supplementary feeding of cleaner fish deployed within commercial salmon pens is necessary to maintain the nutritional condition, welfare and efficacy of the biological controls over the Atlantic salmon grow-out cycle typically lasting 18 to 22 months. Therefore, a feed source adapted to the species grazing feeding habit and to the salmon net-pens rearing environment has first to be developed.

Fresh seafood (e.g. locally collected crushed blue mussels) and manufactured extruded pellets formulated to labrids requirements have been offered using submerged nets and video evidences of consumption were obtained. However, the provision of sufficient fresh

seafood is logistically prohibitive while raising biosecurity concerns. Manufactured dry-pellets (extruded) delivered in fine mesh bags were found to disintegrate within hours in water leading to significant wastage further compromising validation and quantification of feed intake. A practical feed for cleaner fish within salmon net-pens should combine a manufactured base providing a complete and standardised nutrient profile, biosecurity and ease of procurement with high water stability for distribution as grazing substrate.

Hydrocolloid agents have been used as binders to produce practical aqua-feed with high water stability mostly for slow-feeding, i.e. grazing crustaceans and echinoderms (Tacon, 1987). Gelatine (Panreac® Aditio 80-100 Blooms) used to bind frozen shrimp and squid was found palatable and not to compromise growth rate and feed conversion in the common octopus (*Octopus vulgaris*; Quintana et al., 2008). Gelatin at a concentration of 20 to 30 g/L was deemed a suitable binder of microbound diet for barramundi (*Lates calcarifer*) larvae (Partridge and Southgate, 1999). Natural gum extracted from seaweed such as agar, alginate and carragennan have also been successfully applied (Teshima et al., 1984; Cho et al., 1985). Caltagirone et al. (1992) reported better binding performance of agar compared to gelatine, carboxymethyl cellulose and sodium alginate. In another study, agar prevented the disintegration of a manufactured diet for up to six days (Fabbrocini et al., 2012) and had positive effects on the growth rate of crustaceans at inclusions of 20 to 30 g/L (Palma et al., 2008; Volpe et al., 2008; Coccia et al., 2010). However contrasting comparative performances of various binders have been reported across studies which may be due to the type of feed used, variations in the formulation and assessment of the practical feed (Ruscoe et al., 2005; Paolucci et al., 2012). Gelatine is derived from the collagen extracted from the bone and skin of terrestrial and aquatic animals, it is rich in amino acids, odourless and tasteless (Paolucci et al., 2012). Gelatine is soluble in hot water, typically used at concentration over 5 g/L and forms a thermo-reversible gel which

increases in viscosity when cooled below 25 °C (Karim and Bhat, 2009). Agar is a polysaccharide composed of agarose and agaropectine extracted from agarophyte seaweed (Usov, 1998). It dissolves at water temperature above 90 °C, forms a gel at concentrations as low as 3 g/L and is typically used at 20 to 30 g/L in aqua-feed (Fabbrocini et al., 2012). The gelling point of agar solution is lower than its melting point (hysteresis) conferring good gel strength at ambient temperature. The aim of this study was to develop a water stable and well accepted cleaner-fish feed based on a commercially available dry-feed with the view to facilitate feed management and sustain the nutritional condition and welfare of cleaner fish in commercial salmon net-pens.

2. Materials and Methods

2.1 Water-gel strength

Three binders selected based on apparent efficacy and practicality were used: Beef gelatine (BG; 200-250 g high Bloom strength, Dr Oetker(UK)^{Ltd}, Leeds, UK), fish gelatine from cold water fish skin (FG; 90-110 g low Bloom-strength; G7765, Sigma-Aldrich, Dorset, UK) and agar-agar (AA; Special Ingredients^{Ltd}, Chesterfield, UK). The water gel strength of each binder was tested in duplicate under standard conditions, at two temperatures (5 and 20 °C) and over a range of concentrations (n = 7 concentrations / binder) selected according to the general recommendations of each product.

Stock solutions of BG (40 and 50 g/L), FG (150 and 200 g/L) and AA (25 and 40 g/L) were prepared as follow. Binders were weighted (± 0.001 g) and transferred into tap-water (pH: 6.9; 35 °C). BG and FG were kept at 35 °C under constant stirring until dissolution within 10 min. AA was left to hydrate for 10 min at 35 °C prior to boiling for 2 min. Stock solutions were then serially diluted, transferred into 100 ml vials and left to solidify for 24 to 28 h at 5 °C. Gel strength was measured at 5 °C (immediately after refrigeration) or at

20 °C (after 4 h of room conditioning) on 2 vials / temperature / concentration / binder using an in-house piston system. Pressure was applied at 10 g/cm^2 increments until breakage of the gel surface which was recorded as a quantitative measure of gel-strength.

2.2 Water stability of the experimental feed

Binder solutions showing satisfactory water-gel strength (AA 10 and 20 g/L, BG 50 g/L) were selected for preparation of the experimental feed using two types of complete dry-feed components: manufactured extruded pellets (EP; Ø 14 mm Symbio wrasse maintenance; BioMar(UK)^{Ltd}, Grangemouth, UK) and its corresponding grinded ingredient mix provided by the manufacturer (IM; Symbio marine mix). The water stability of each formulation was tested within standardised "hanging" feeders (Fig 1a) prepared as follow. Binder solutions were prepared as previously, EP were then mixed with an excess of binder solutions for 20 min while IM was mixed with either 1.6 or 2.5-fold its weight in volume of binder solution (IM + binder solution at 1/1.6 or 1/2.5 (w/v) ratio) until a consistent texture was achieved. Each formulation was then transferred into a hanging feeder left to set for 24 to 28 h at 5 °C prior to immersion. Upon setting, the EP feed consisted of a single block of pellets within a semi-translucent gel matrix while the IM feed consisted of a single gel block of homogenous texture and colour. For each experimental feed, duplicate feeders were placed for 6 days within a 20 L freshwater tank at 18 °C with an aquarium pump set at 2 L/min to create a consistent water flow. The wet-weight (± 0.01 g) of feed within each feeder was determined prior to immersion and at 24 h intervals over the 6-day trial to quantify feed-loss. Upon completion, water stability tests were repeated on the formulation found water stable but kept frozen at -20 °C for a week after preparation.

151 *2.3 Farmed ballan wrasse feeding trial in commercial tanks*

152 Two feeds were selected according their stability to be tested for palatability (IM + 20
153 g/L AA solution at 1/1.6 and 1/2.5 (w/v) ratio; IM 1.6 and IM 2.5 respectively).
154 Experimental feed were prepared within standardised hanging feeders as previously and
155 deployed within a flow-through tank system (3 circular tanks, Ø 1.5 m, 1 m depth; Otter
156 Ferry Seafish^{Ltd}, Tighnabruaich, UK) supplied with natural seawater (11.0 ± 0.1 °C; 35.0 ±
157 0.5 ppt) and maintained under natural light. Two tanks were stocked with farmed ballan
158 wrasse (1760 – 2050 wrasse / tank; 51.8 ± 1.3 g; 14.1 ± 0.1 cm) previously fed on extruded
159 pellet (wrasse grower Ø 2 mm; BioMar(UK)^{Ltd}, Grangemouth, UK) using an automatic
160 belt-feeder. The third tank was identical but kept without fish as control environment. At
161 the start of the trial automatic feeding was discontinued, the two wrasse tanks each
162 simultaneously received an IM 1.6 and an IM 2.5 hanging feeders while the control tank
163 received duplicate hanging feeders of both formulations (n = 2 feeders / formulation with
164 and without wrasse). The net-weight of feed within each feeder (± 0.01 g) was measured
165 prior to deployment and at 24 h intervals during 6 days when the trial was terminated.
166 Underwater video recordings were performed to further confirm feed intake.

167

168 *2.4 Wild wrasse feeding trial in commercial salmon net-pens*

169 The preferred jelly-feed formulation (IM 1.6) was deployed within commercial salmon
170 net-pens (25 m * 25 m square pens, 16 to 20 m depth; Marine Harvest(Scotland)^{Ltd}, loch
171 Leven, Ballachulish; UK) stocked with Q1 2014 Atlantic salmon (n = 57545 ± 547) and
172 wild wrasse captured in June and over the trials duration in July-August (1.2 to 2.1 wrasse:
173 salmon during the trial). No supplementary feed was provided before the experiment. The
174 wild wrasse population comprised ballan (57.9%; 78.2 ± 5.8 g; 167 ± 3 mm), goldsinny

175 (*Ctenolabrus rupestris*; 29.9%), corkwing (*Crenilabrus melops*, 7.6%), rockcook
176 (*Centolabrus exoletus*, 3.7%) and cuckoo (*Labrus mixtus*, 0.9%) wrasse.

177 During the first period (2 weeks), two salmon net-pens each received 2 hanging feeders
178 prepared as previously and 2 "tray" feeders (Fig 1b) holding formulation IM 1.6 and
179 placed within a "wrasse shelter" (Fig 1c) located at a pen corner as follow. Within the
180 shelter, two different feeders were placed in duplicate, at 11 and 12 m depth. The same
181 experimental set-up was performed in an additional commercial cage stocked with salmon
182 but without wrasse over two consecutive weeks. Immediately upon completion of period 1,
183 the second period (4 weeks) tested the effect of feeder location (within a wrasse shelter or
184 within a feeding shelter) on feed intake using the preferred feed and feeder type (IM 1.6;
185 hanging feeder). The two commercial pens stocked with wrasse received four feeders each,
186 2 feeders placed within the wrasse shelter as previously (11 and 12 m depth) and 2 feeders
187 placed within a feeding shelter (Fig 1d) located immediately below at 13 and 14 m depth.
188 Over both periods the feeders were changed every week weighting the feed before and
189 after water immersion. Water temperature and salinity averaged 11.6 ± 0.1 °C and 33.9 ±
190 0.1 ppt over the period.

191

192 *2.5 Nutritional quality*

193 Feed samples (IM 1.6) were prepared as previously including overnight setting at 5 °C
194 then kept refrigerated (5 °C; 2 days) or frozen (-20 °C; 7 days) prior to immersion in 11 °C
195 natural seawater (Circular flow-through tank; Otter Ferry Seafish^{Ltd}). Feed samples were
196 taken at 1 cm depth of the feed surface layer immediately prior and after 1 day and 8 days
197 of immersion, air-seal packed and immediately frozen at -20 °C until analysis in
198 comparison to the manufactured dry IM. Moisture content was determined by thermal
199 drying to constant weight at 110 °C for 24 h (Method 930.15; AOAC 2000) and crude

protein by Kjeldahl analysis (Persson, 2008). Total crude fat was quantified by Soxhlet extraction using petroleum ether after pre-treatment by acid hydrolysis (ISO 11085:2008 (B)) and ash by dry ashing in a muffle furnace at 600 °C overnight (AOAC 2000; method 942.05). Total lipid fatty acid analysis was determined according to AOCS (2013; method Ce 1i-07 – fatty acids) as described in Bell et al. (2010) by Folch extraction and fatty acid methyl esters quantification by gas-liquid chromatography. TBARS analysis was performed as described in Mourente et al. (2000). All analyses were performed by the Nutritional Analytical Service of the Institute of Aquaculture (Stirling, UK) on duplicated assay of duplicated samples.

2.6 Statistical analysis

Datasets were checked for normality using the Kolomogorov-Smirnov test and for homogeneity of variance using Levene's tests and observations of residual plots; proportions were arc-sine transformed. The effect of temperature and binder on gel-strength was tested over the common range of test concentrations using a non-parametric repeated measure ANOVA (Friedman-test) with a Dunn's Multiple Comparisons Test. Repeated measure ANOVA manipulated by GLM were used with a Tukey post hoc test when significant differences occurred to test the effect of time on the mean total-weight of each formulation or feeder type and to test the effect of formulation with and without wrasse on the relative left-over feed weight across time-points (replicate tanks and feeders pooled). In the sea pen trial, the effect of time and feeder type on the weekly mean feed-intake per feeder (n = 2 feeder / feeder type/ time-point / pen) was tested with replicate pens nested within treatment grouping by a two-way ANOVA manipulated by GLM with a Tukey posthoc test when significant differences occurred. Parametric tests were performed

using Minitab v.16 and non-parametric using Instat v.3.01.32 with a significance level of 5% ($P < 0.05$). Results are presented as mean ± SE.

3 Results

3.1 Water-gel strength

Gel strength increased with binder concentrations and was visibly but not significantly higher at lower gel temperature for all binder tested (Fig 2). Over the range of identical concentrations tested (10 to 40 g/L inclusive), AA gel was significantly stronger than FG and BG gels with no temperature effect (repeated measure ANOVA). AA showed jellifying properties from a concentration of 2.5 g/L at both temperatures and the gel-strength reached over 1000 g/cm^2 at a concentration of 20 g/L (1239 ± 0 g/cm^2 and 1114 ± 0 g/cm^2 at 5 and 20 °C respectively). In comparison, BG solution formed a gel from a concentration of 25 g/L at 5 °C and the maximal gel strength measured in this study remained low (141 ± 0 g/cm^2 and 61 ± 0 g/cm^2 at 5 and 20 °C respectively). FG formed weak gels only at low temperature (5 °C) and high inclusion rates of 150 g/L (25 g/cm^2) and 200 g/L (61 g/cm^2).

3.2 Saturation of the dry-feed component

Prior to testing the water stability of the experimental feed, absorption tests were performed to establish the preparation protocols and determine the possibility to saturate the extruded pellets (EP) with binder solutions against water ingress upon immersion. The water absorbance capacity of the supplied EP was 3.07 ± 0.02 mL/g. When placed in an excess of a 50 g/L BG, 20 g/L AA and 10 g/L AA liquid solutions, the EP reached 44.4 ± 0.8 %, 34.0 ± 1.0 % and 35.2 ± 0.7 % respectively of its water absorbance capacity (triplicated tests). In all cases, a residual dry-core was observed upon sectioning of the

pellets. Difficulties in saturating the pellet with viscous binder solutions motivated the use of the grinded dry ingredients mix (IM; as used by the manufacturer to produce the EP tested) as dry-feed component of the experimental feed. Absorption tests documented that a solution`s volume of 1.6 and 2.5 fold the IM weight was required to fully moist the feed and first observe an excess of solution respectively.

3.2 Water stability of the experimental feed

When using 50 g/L BG, both IM and EP based feed showed a significant weight loss after 2 days (- 19.4 ± 0.3 %) and 3 days (- 11.8 ± 0.7 %) of immersion respectively (Fig 3). Feed prepared using 10 g/L AA as binder of EP or IM both showed a loss in weight first significant after 4 days of immersion. When binding EP with 20 g/L AA, feed loss was not significant after 6 days of immersion (- 2.3 ± 0.3 %) but a loss of content from pellets swelling and breakage was evident (Fig 1a). In comparison, the relative-weight of the experimental feed combining IM and the 20 g/L AA binder at 1/1.6 and 1/2.5 (w/v) ratio did not significantly vary after 6 days of immersion and when deployed either fresh or following a week of -20 °C freeze preservation (+ 0.8 ± 0.2 % to + 2.9 ± 1.8 %; Fig 3).

3.3 Farmed ballan wrasse feeding trial in commercial tanks

The weight of each experimental feed (IM + 20 g/L AA at 1/1.6 and 1/2.5 (w/v) ratio; IM 1.6 and IM 2.5 respectively) deployed in sea-water within the control tank (no wrasse, no salmon) did not vary over time (Fig 4). Across time-points, the relative left-over feed weight was significantly lower when deployed with wrasse than in the control tank (IM 1.6 = 42.6 ± 11.3 % and 101.6 ± 0.11 % respectively; IM 2.5 = 73.9 ± 10.2 % and 102.4 ± 0.2 % respectively). In both wrasse tanks, a reduction in the relative-weight of feed was evident within two days (-7.7 ± 2.2 %) and all feeders were emptied after 7 days of

deployment (Fig 4). In addition, IM 1.6 was always first to be fed upon and fully consumed following which the bulk of IM 2.5 was ingested.

3.4 Wild wrasse feeding trial in commercial salmon net-pens

During period 1, the mean total-weight of both types of feeders (hanging and tray feeders; n = 2 feeders / feeder type / week; IM 1.6 only) significantly increased after one week of immersion in the control pen (+3.1 ± 0.1 % and + 7.9 ± 0.9 % of the initial-weight, hanging and tray feeders respectively). When deployed in pens stocked with wrasse, the increase in mean total-weight was significant for the tray feeders (+8.2 ± 0.7 % of the initial-weight) but not for the hanging feeders (+1.0 ± 1.3 %). Feeding marks were observed on one hanging feeder along with a feed intake of 52 g (- 8.1 % of the initial-weight) during week 2 (Fig 5a.). During the second period (week 3 to 6; Fig 5b), there was an overall significant effect of time and treatment on mean feed intake per feeder ($P < 0.001$; 2-way ANOVA) being significantly higher from feeders located within the feeding shelter than within the wrasse shelters (72.6 ± 4.0 % and 27.4 ± 4.0 % of the total-weekly feed intake respectively). However due to a significant effect of replicate pens within each treatment; no significant differences between treatments at each time point were observed except at week 4.

3.5 Nutritional quality

The moisture content of the selected formulation (IM + 20 g/L AA at 1/1.6 (w/v) ratio) was significantly higher than that of the grinded used as dry component (Table 1). Mean oil, protein and ash content in the experimental fed were lower by a factor of 2.33; 2.37 and 2.28 respectively compared to a 2.60 dilution factor from water incorporation at preparation. When expressed in dry-weight equivalent, there were no significant difference

299 in oil and protein content between the IM and the experimental feed preserved fresh (5 °C;
300 2 days) or frozen (-20 °C; 7 days) after preparation and sampled after up to 8 days of
301 immersion. Fatty-acid profiles did not vary between the supplied IM and the experimental
302 feed sampled after preparation (data not shown). The concentration of TBARS was on
303 average 12.9 ± 0.5 % higher in the experimental feed with significantly higher levels
304 measured on feed sampled after preparation and 1 but not 8 days after immersion.
305
306 **4. Discussion**
307 The study developed and validated a practical aqua-feed for ballan wrasse deployed in
308 Atlantic salmon net-pens that met the primary requirements of being well accepted by the
309 wrasse, physically water-stable for 1-week with no nutrient leaching and based on a dry-
310 manufactured component. The comparative gel strength test clearly rejected gelatin from
311 cold-water fish skin (FG; 90-110 g Bloom) as a potential binder in the current application.
312 Low gel strength and melting temperatures are indeed characteristic of cold-water fish
313 gelatin (Gómez-Guillén et al., 2002). High bloom gelatin (BG; 220-230 g Bloom) in the
314 form of BG formed a gel from a concentration of 40 g/L at the highest temperature tested
315 (20 °C) demonstrating a potential as aqua-feed binder as previously reported (Partridge and
316 Southgate, 1999; Quintana et al., 2008; Simon, 2009). However, AA generated a
317 significantly stronger gel with e.g., the 20 g/L AA water-gel measured to be 16-fold
318 stronger than the 50 g/L BG gel at 20 °C under standard test conditions. The AA water-gel
319 strength measured in this study (640 ± 10 g/cm^2 at 15 g/L; 20 °C) was marginally inferior
320 than previously reported under similar conditions (700 to 1000 g/cm^2; AgarGel, 2014)
321 which could be due to different agar quality grades, water quality or methodologies
322 applied. Overall, this trial identified 50 g/L BG, 20 g/L AA and 10 g/L AA as potential
323 binding solutions for the development of a water stable aqua-feed.

The experimental feed using 50 g/L BG as binder of extruded pellets or grinded ingredients mix partially or fully disintegrated within 6 days of immersion at 18 °C. When using AA, a degree of feed loss occurred only with EP as dry-component. This was clearly due to the swelling of the outer most pellets resulting in the breakage of the surrounding gel matrix which was deemed to originate from a lack of saturation of the pellets by the binder. In contrast, full water stability was achieved using 20 g/L AA as IM binder incorporated at either 1/1.6 or 1/2.5 (w/v) ratio and deployed fresh or following 1-week of freeze preservation at -20 °C. This is in accordance with the study by Fabbrocini et al. (2012) who successfully used 30 g/L AA to stabilise homogenised algae and manufactured pellets grind for 5 days in 20 °C seawater. Although previously suggested (Fuchigami and Teramoto, 2006), freeze preservation of the agar-IM based experimental feed did not compromise water stability in this study thereby opening the possibility to freeze preserve the cleaner-fish diet for logistic purpose.

Palatability of the experimental feed found to be water-stable for 1-week (IM binded with 20 g/L AA) was confirmed by visual observation within 48 h of deployment with farmed ballan (https://vimeo.com/102023728) and a reduction in the relative-weight of the feed offered in the wrasse tanks but not in the control tank. In both wrasse tanks, preferential feeding on the formulation holding a lower ratio of 20 g/L AA solution (1/1.6 compared to 1/2.5; w/v) was found. The softer texture achieved at lower binder inclusion rate could increase palatability, facilitate grazing or the release of attractants. This formulation further presents the advantage of being more energy dense by design hence was selected for final field-testing and nutritional analysis. Deployment of the selected formulation in a commercial salmon net-pen without wrasse confirmed water stability at sea in addition to the non-consumption by salmon. Presentation of the feed within a static feeder was indeed expected not to attract Atlantic salmon described as visual feeders on

motile prey (Jacobsen and Hansen, 2001). Wild wrasse first accepted the feed 1 to 2 weeks after deployment which was deemed a short acclimation period for wild wrasse never previously exposed to a manufactured diet and stocked at low density in a net-pen environment. Feed consumption was first observed from the hanging feeder which was selected as preferred distribution method also as the tray feeder was rapidly spoiled by organic waste. Subsequently and over the second experimental period, total feed intake from hanging feeders progressively increased in both experimental pens and preferential feeding from the feeding shelter at 13 and 14 m than within the wrasse shelter at 11 and 12 m was documented. Although the effect of depth and location could not be discriminated, preferential feeding away from the wrasse shelter has practical benefits as the feeding shelter was easily handled while allowing leaving the wrasse shelter undisturbed.

On a wet-weight basis, nutritional components were diluted by a factor of 2.29 ± 0.06 on average which was highly consistent across parameters and samples. However, a dilution factor of 2.6 would have been expected from the preparation protocol indicating a degree of water evaporation during boiling of the agar solution which should therefore be accounted for during implementation. On a dry-weight basis, nutrient analysis indicated an absence of macronutrients leaching after up to 8 days of immersion and including on freeze preserved feed in line with the findings of Simon (2009). Future studies should assess the loss of micronutrients such as water-soluble vitamins found to be significant in an alginate microbound diet for the freshwater prawn *Macrobrachium rosenbergii* (Kovalenko et al., 2002). In addition, a constant FA profile was measured indicating an absence of significant lipolysis and fatty acid deterioration from hydrolysis or thermolysis following preparation, preservation and deployment of the proposed cleaner-fish feed. Similarly, no effect of heat exposure during pelletisation nor of 45 days freeze preservation was observed on the fatty acid profile of a fish-oil based rodent diet containing antioxidant (Lytle et al., 1992) while

a temperature of 180 °C was suggested not to induce thermal degradation of the more susceptible long-chain polyunsaturated fatty acids of fish oil (Fournier et al., 2006). However, TBARS concentration was significantly higher in the experimental feed than in the ingredient mix at few time-points suggesting that the feed preparation protocol could induce a degree of lipid peroxidation. This suggests the need to reinforce the antioxidant profile of the ingredient mix using, e.g., butylated hydroxytoluene (BHT). Together, the positive results obtained warrant further assessment of the dietary qualities of the jelly-feed developed including shelf-life optimisation and the potential impact of agar inclusion on gut health and nutrients digestibility in ballan wrasse.

An aqua-feed prepared by mixing a manufactured grinded ingredient mix with a 20 g/L agar solution at a 1/1.6 (w/v) ratio is proposed as the basis of a novel cleaner fish feed adapted to the grazing behaviour of wrasse within the salmon sea-pen environment. The proposed aqua-feed combines the complete nutrient profile of a manufactured diet with high water stability to minimise feed loss and facilitate the supplementary feeding of cleaner fish stocked in salmon from weekly interventions. This aqua-feed allows validating and quantifying feed intake providing a valuable indicator of survival, health and activity of the biological stock. The feeding strategy must now be established including feeding quantities and frequencies according to e.g. water temperature and sea-lice pressure in order to secure the health and delousing activity of the biological stock over the salmon rearing cycle.

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494

FIGURE LEGENDS

Figure 1: Pictures of (**a.)** a standardised PVC hanging feeders (Ø 55 mm; 235 mm length, 500 mL effective volume) with four circular side openings (Ø 60 mm) filled with experimental feed (left to right) IM + AA 20 g / L at 1:1.6 (w : v) ratio and EP + AA 20 g / L. Pictures were taken after 6 days of water immersion during the "water stability trial"; (**b.)** "tray" feeders (20 x 30 cm sides, 3 cm high, 1200 mL effective volume); (**c.)** wrasse shelter (1 m Ø; 2 m high) with both types of feeders presented adjacent and at two levels of the shelter; (**d.)** two hanging feeders each placed within a feeding shelter (30 cm diameter weighted ring with 50 cm long artificial kelp). Pictures b., c., d.: Wild wrasse feeding trial in commercial salmon net-pens.

Figure 2: Pressure required to break the surface of water-gels made using different concentrations of fish gelatine (FG), beef gelatine (BG) and agar-agar (AA), tested at 5 and 20 °C under standard conditions. The gel-strength of AA was significantly higher over the range of identical concentrations tested (10 to 40 g/L inclusive, no significant temperature effect; non-parametric repeated-measure ANOVA, Friedman-test). Values are presented as mean ± SE with n = 2 / concentrations / temperature.

Figure 3: Proportion of left-over feed relative to their initial-weight when immersed in 18 °C freshwater for 6 days. Experimental feed were prepared using solutions of 50 g/L beef-gelatine (BG), 10 and 20 g/L agar-agar (AA) as binders of extruded pellets (EP) or of the corresponding grinded ingredient mix (IM) at an IM/binder solution ratio of 1/1.6 or 1/2.5 (w/v). Experimental feed were tested fresh or after freeze-storage at -20 °C for 1 week (frozen). Asterisks denote significant differences in total-weight compared to the initial value within each feed formulation (One-way repeated measure ANOVA). Values are presented as mean ± SE with n = 2 / formulations. Note: broken y-axis.

Figure 4: Proportion of left-over feed relative to their initial-weight when immersed in flowing 11 °C seawater for 7 days with (tank 1 and 2) or without (control tank) farmed ballan wrasse. IM 1.6 and IM 2.5: Experimental feed prepared using the grinded ingredient mix and 20 g/L of agar-agar solution at a ratio of 1/1.6 or 1/2.5 (w/v) respectively.

Figure 5: Mean weekly feed intake per feeder by wild wrasse in commercial salmon pens with feed offered (**a.**) in hanging or tray feeders (n = 2 feeders / feeder type / pen) all located within a wrasse shelter (week 1 and 2; period 1) and (**b.**) from hanging feeders located either within a wrasse shelter or within a feeding shelter (n = 2 feeders / location / pen; week 3 to 6, period 2). Over period 2; mean feed intake was significantly higher from the feeding than from the wrasse shelters. Different letters denote significant differences in mean feed intake per feeder between time-points, treatments and replicates within period 2 (Two-way ANOVA).

Table 1: (**a.**) Proximate composition (wet-weight basis) (**b.**) proximate composition (dry-weight basis) and (**d.**) thiobarbituric acid reactive substances (TBARS) concentration of the supplied grinded ingredient mix (IM) and of the proposed experimental feed preserved refrigerated (2 days; 5 °C) or frozen (1 week; -20 °C) after preparation and sampled immediately prior, 1 or 8 days of immersion in 11 °C seawater. Values are shown as mean ± SE of duplicated samples assayed in duplicate. Different superscript letters indicate significant differences between experimental groups.

	Grinded ingredient mix (IM)	**Experimental feed (IM + 20 g/L agar solution at 1/1.6 (w: v) ratio**					
		Prior to immersion		**Day 1 post-immersion**		**Day 8 post-immersion**	
		Fresh	**Frozen**	**Fresh**	**Frozen**	**Fresh**	**Frozen**
a. Proximate composition (g / 100 g wet-feed)							
Moisture	8.2 ± 0.0^{a}	60.1 ± 0.5^{b}	59.3 ± 0.4^{b}	60.0 ± 0.3^{b}	63.1 ± 0.3^{c}	63.6 ± 0.4^{c}	63.1 ± 0.4^{c}
Oil	10.5 ± 0.1^{a}	4.7 ± 0.1^{b}	4.6 ± 0.2^{b}	4.5 ± 0.1^{b}	4.0 ± 0.1^{b}	4.4 ± 0.2^{b}	4.7 ± 0.2^{b}
Protein	55.4 ± 0.2^{a}	25.8 ± 0.4^{b}	24.3 ± 0.3^{bc}	23.8 ± 0.2^{c}	22.5 ± 0.4^{cd}	22.0 ± 0.3^{d}	22.0 ± 0.6^{d}
Ash	9.5 ± 0.0^{a}	4.2 ± 0.0^{cd}	4.0 ± 0.0^{e}	4.1 ± 0.0^{dc}	4.2 ± 0.0^{cd}	4.3 ± 0.0^{bc}	4.3 ± 0.0^{b}
b. Proximate composition (g / 100 dry-feed)							
Oil	11.4 ± 0.1^{ab}	11.8 ± 0.2^{ab}	11.2 ± 0.4^{ab}	11.3 ± 0.3^{ab}	10.9 ± 0.3^{a}	12.1 ± 0.5^{ab}	12.7 ± 0.5^{b}
Protein	60.4 ± 0.2^{ab}	64.5 ± 1.0^{b}	59.7 ± 1.4^{a}	59.5 ± 0.7^{a}	61.1 ± 0.6^{ab}	60.4 ± 0.4^{ab}	59.6 ± 1.9^{a}
Ash	10.4 ± 0.1^{a}	10.5 ± 0.1^{a}	9.9 ± 0.1^{a}	10.3 ± 0.1^{a}	11.3 ± 0.2^{b}	11.8 ± 0.1^{b}	11.7 ± 0.1^{b}
c. TBARS (mg malonaldehyde / kg dry feed)							
	4.14 ± 0.01^{ab}	5.05 ± 0.01^{c}	5.03 ± 0.10^{c}	4.72 ± 0.03^{bc}	5.05 ± 0.01^{c}	4.58 ± 0.12^{ab}	3.62 ± 0.31^{a}