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Towards sustainable feeds for abalone culture: Evaluating the use of mixed species seaweed meal in formulated feeds for the Japanese abalone, *Haliotis discus hannai*

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ABSTRACT

Given the growing importance of the aquaculture sector in contributing to global seafood production, there needs to be a concerted effort to develop commercially relevant and ecologically sustainable feeds for the cultivation of commercially important species. Limitations on the availability and use of fish meal through EU directives (EC Regulation 999/2001 & EC Regulation 183/2005) are focussing research efforts to find alternative sources of protein for aquaculture feeds. Marine algae, although low in protein, are widely being considered as a potential alternative to fish meal as not only do they have the potential to impart additional health benefits when ingested but they can also be cultured under enrichment conditions to enhance their nutritional profile as a feed additive. For abalone (a marine gastropod mollusc), several studies have highlighted the benefits of a diet of fresh mixed species seaweed for cultivation. However, the performance of a mixed species marine algae meal, consisting of *Laminaria digitata* meal, *Palmaria palmata* meal and *Ulva lactuca* meal, in a formulated diet for abalone has not apparently been investigated. In this study, the performance of five novel, isonitrogenous, konjac glucomannan–xanthan gum (KX) bound feeds for the abalone *Haliotis discus hannai* were evaluated in a 12-week experimental growth trial. Comparisons were made between a basal diet formulation (Diet A), a basal diet + lipids + choline chloride (Diet B) and diets containing mixed seaweed meal (Diet C) or fish meal (Diet D) or mixed seaweed meal + fish meal (Diet E). Freshly harvested *L. digitata* was included as a natural feed type and experimental control. Dry matter leaching was assessed and no significant differences in the dry matter loss over 3 and 4 days were observed between the experimental KX feeds, although *L. digitata* was a significantly more seawater stable feed type. Daily food consumption (DFC) of Diet C and Diet E was significantly lower than that of fresh *L. digitata* and the DFC of Diet D was significantly higher than that of *L. digitata*. Diet E had significantly higher food conversion efficiency than *L. digitata*, Diet A, Diet B and Diet D. There was no difference in the protein efficiency ratio (PER) between the formulated diets but the PER of Diet D was significantly different to *L. digitata*. *L. digitata* had significantly higher linear growth rates than Diet B, and significant differences in the specific growth rates were observed between Diet A and Diet D only. No significant differences in body weight to shell length ratios and percentage survival between treatments were observed. This study highlights the potential for a mixed species seaweed meal as a fish meal replacement in formulated feeds for abalone.

1. Introduction

The premium market status of abalone worldwide coupled with over-exploitation in many traditional natural harvest locations has intensified efforts towards commercial viability and sustainability in this aquaculture sector (Cook and Gordon, 2010). Although formulated feeds are available, several of the binders (e.g. agar, carrageenans and alginates) and traditional component ingredients (e.g. fish meal and casein) previously evaluated to give good feed performance (Fleming et al., 1996; Pearce et al., 2002; Sales, 2004) are too costly for commercial scale feed production and may be ecologically unsustainable in the long term. High feed production costs have been exacerbated by exorbitant importation costs to many peripheral locations, where many aquaculture activities are located. Furthermore, one of the foremost feed ingredients recommended to achieve commercially relevant growth rates, fish meal, is currently a controlled substance within the European Union (EC Regulation 999/2001 & EC Regulation 183/2005) and statutory limitations are imposed on its availability and use. The feasibility of commercial scale abalone cultivation using commercially available abalone feeds is generally seen as an unviable option for local producers in Ireland where an alternative natural resource, kelp, is relatively inexpensive by comparison. However, the use of locally harvested kelp for

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commercial scale abalone culture in Ireland continues to put pressure on these natural marine habitats. Overharvesting of the kelp beds in these areas leads to a loss in biodiversity and the time to regenerate, even under sustainable harvesting regimes, can result in slow recovery rates (Kelly, 2005; Werner and Kraan, 2004).

The commercial production of abalone in Ireland is an ideal target industry for the development of artificial feeds for all life history stages because the grow-out technology in Ireland is entirely land-based. This contrasts with other regions, such as South Africa and Australia, where ranching is permitted. The fact that commercial scale production of abalone can be maintained through the use of kelp as the sole feed option has resulted in some research efforts to develop an artificial feed that resembles this natural resource. Such a feed would not only encompass sufficient nutritional components to support growth and sustain survival, with a feed conversion ratio (FCR) of commercial interest, but would also represent a move towards an ecologically sustainable feed with minimal potential impacts on the local environment. The feed would also provide a consistent product for abalone cultivation since the quality and nutritional composition of wild seaweed varies seasonally (Haug and Jensen, 1954). Previous studies have found that growth of the perlemoen abalone, *Haliotis midae*, was maximised on a mixed natural diet of *Gracilaria gracilis*, *Ulva lactuca*, and kelp (*Ecklonia maxima*) (Naidoo et al., 2006) and a study conducted by The California State University and the Monterey Abalone company has shown that 25% greater growth rates of red abalone, *Haliotis rufescens*, have been achieved with 5% dietary supplementation of three red algae species, *Gracilariopsis andersonii*, *Palmaria mollis*, and *Chondracanthus corymbiferus*, in a fresh kelp diet (Frazer, 2009; Personal communication Prof. Michael Graham, California State University). Zhanhui et al. (2010) achieved increased growth in shell length and improved feed conversion efficiency in cultured *Haliotis discus hannai* fed a mixed diet of fresh kelp, *Laminaria japonica*, and the red algae *Gracilaria lemaneiformis*. However, the use of mixed algae in meal form in a formulated diet has not been reported in the literature.

The hydrocolloid binder complex, konjac glucomannan–xanthan gum (KX) has previously been used in other areas but its use in the production of aquaculture feeds is relatively new (O'Mahoney et al., 2011). KX gels have been produced at total polysaccharide levels of only 0.02%, which is the lowest gelling concentration observed for a carbohydrate system (Dea, 1993). They are relatively inexpensive to produce and production can be scaled to suit small-scale systems. The KX gelling system forms strong cohesive gels which physically entrap the feed particles in the gel network. Any interactions between the gel network and the components contained within the feed would result in the compromised formation of this network and therefore the evaluation of dry matter leaching from the experimental feeds is an important consideration for feed development. A previous study has indicated that formulated feeds produced using the KX binder configuration of seawater (SW) 2% KX; 1:1 were particularly suitable for further formulated feed development for the abalone *H. discus hannai* (O'Mahoney et al., 2011).

The nutritional requirements of abalone in culture have been well studied. Diet composition, based on a comprehensive review of the literature, aimed to achieve a target protein concentration of 35% as this approximate value has been recommended for *H. discus hannai* (Mai et al., 1995a) and *H. midae* (Sales et al., 2003). Target protein concentrations were achieved through the use of several feed ingredients; soy protein isolate (89% P), derived from soybean, which is an ingredient that is both highly digestible (Sales and Britz, 2002, 2003) and produces high growth rates (Uki et al., 1985), *Laminaria digitata* meal for improved feed palatability and attractant properties, a mixed seaweed meal [consisting of *L. digitata* meal (12.56% P), *Palmaria palmata* meal (18.77% P) and *U. lactuca* meal (7.35% P) in the ratio of 1:1:1] and low temperature processed fishmeal which has been shown to result in improved feed attraction, palatability and growth rates (Britz, 1996a; Uki et al., 1986a; Viana et al., 1996). The diets were supplemented with choline chloride, a water-soluble vitamin (B_4) (Hertrampf and Piedad-Pascual, 2000) which is involved in a number of key physiological processes (Zeisel, 1992) and has been supplemented in formulated diets for abalone in several growth studies (Britz, 1996a, 1996b; Durazo-Beltran et al., 2004; Garcia-Esquivel and Felbeck, 2006; Garcia-Esquivel et al., 2007; Gomez-Montes et al., 2003; Mai et al., 1995a, 1995b; Tan and Mai, 2001; Tan et al., 2001; Zhu et al., 2002). The main aim of this study was to evaluate a mixed species seaweed meal in a composite diet formulation for *H. discus hannai* when used alone or in combination with fish meal. In order to contextualise the impact of the component feed ingredients on the KX-binder matrix, a dry matter leaching experiment was conducted at the end of the experiment.

2. Materials and methods

The formulation for the basal abalone diet and the methodologies for feed preparation, the experimental culture system and experimental design have been previously described in O'Mahoney (2009) and O'Mahoney et al. (2011). In brief, the pre-mixed dry ingredients and lipids were added to a hot, viscous konjac glucomannan–xanthan gum solution and mixed to a uniform consistency for 3 min. The hot feed was poured into a rigid food storage container, covered with a lid, cooled rapidly and refrigerated overnight. On the following day the feed was sliced into strips and air-dried in a drying room equipped with a convector heater and dehumidifiers for 24–30 h (average temperature 30 °C, average relative humidity 28%). All air-dried feeds were stored in air-tight containers.

2.1. Experimental diets

The following five pre-commercial experimental feeds were assessed in this study:

Basal abalone diet (Diet A)

Basal diet + lipids + choline chloride (Diet B)

Basal diet + mixed seaweed meal + lipids + choline chloride (Diet C)

Basal diet + low temperature fish meal + lipids + choline chloride (Diet D)

Basal diet + mixed seaweed meal + low temperature fish meal + lipids + choline chloride (Diet E).

Soy protein isolate was obtained from The Solae Company (Leper, Belgium), *L. digitata* meal was obtained from Arramara Teoranta (Co. Galway, Ireland), *P. palmata* meal and *U. lactuca* meal were obtained from Seaweed Ireland (Beara, Ireland), choline chloride was obtained from Sigma (Dorset, U.K.), low temperature fish meal and fish oil were obtained from United Fish Industries (Donegal, Ireland, fish meal licence no. FM26/08), limestone flour was obtained from Ballyellen Limestone Flour Works (Kilkenny, Ireland), and vitamins and minerals were obtained from Inform Nutrition (Cork, Ireland). The composition of the experimental feeds is outlined in Table 1.

Proximate analyses were conducted on all experimental diets and a sample of fresh *L. digitata* which was harvested during the experiment. Moisture content was determined by drying the samples in a drying oven at 105 ºC overnight. Total lipid content was determined by the Rafatec method. Total nitrogen was calculated using the Kjeldahl method according to standard AOAC procedure (A.O.A.C., 1990) and multiplied by a factor, 6.25, assuming that 16% of total nitrogen in this feed type is digestible. Ash was determined by incinerating a sample free from organic matter (organic matter burned off by Bunsen burner) at 550 ºC for 8 h. Total carbohydrates were estimated by subtraction. Values obtained for protein, carbohydrate, fat and ash were converted to % dry matter in order to calculate energy values for the diets. The

Table 1
Ingredient composition (g dry matter kg^{-1}), proximate composition (g kg^{-1}) and energy (kJ g^{-1}) and cumulative dry matter leaching (g kg^{-1}; mean ± S.E.; $n = 3$) of the experimental feeds.

		Diet A (abalone base)	Diet B (lipid; choline chloride)	Diet C (mixed weed, lipid & choline chloride)	Diet D (LT FM; lipid; choline chloride)	Diet E (LT FM; mixed weed; lipid; choline chloride)	*Laminaria digitata*
Ingredient composition (g dry matter kg^{-1})	KX binder: SW 2% KX; 1:1	174.7	176.5	172.7	159.6	157.3	–
	Soy protein isolate[1]	352.9	353.6	347.6	202.3	200.00	–
	Laminaria digitata meal[2]	319.7	320.3	103.9	206.7	67.8	–
	Palmaria palmata meal[3]	–	–	117.5	–	76.6	–
	Ulva lactuca meal[4]	–	–	111.5	–	72.8	–
	LT fish meal[5]	–	–	–	203.6	200.3	–
	Limestone flour	107.2	59.3	58.3	140.0	138.4	–
	Vitamins[a]	14.9	14.9	14.7	14.1	13.9	–
	Minerals[b]	30.6	30.6	30.1	29.0	28.6	–
	Choline chloride	–	9.8	9.6	10.0	9.9	–
	Fish oil	–	17.3	17.0	17.4	17.2	–
	Corn oil	–	17.6	17.3	17.4	17.2	–
Proximate composition & energy values	Protein[c]	378.8	387.8	380.8	368.7	377.2	108.2
	Ash[c]	326.9	315.6	283.5	382.4	357.2	226.7
	Carbohydrate[c]	283.5	259.0	300.1	198.2	216.2	627.9
	Fat[c]	10.9	37.5	35.6	50.8	49.4	37.6
	Energy[d]	11.50	12.24	12.74	11.40	11.80	13.74
Cumulative dry matter leaching (g kg^{-1}; mean ± S.E.)	Day 3	240.6 ± 7.5[x]	240.6 ± 7.5[x]	240.6 ± 7.51[x]	240.6 ± 7.5[x]	240.6 ± 7.5[x]	42.5 ± 55.2[y]
	Day 4	268.7 ± 9.0[x]	268.7 ± 9.0[x]	268.7 ± 9.0[x]	268.7 ± 9.0[x]	268.7 ± 9.0[x]	87.0 ± 61.8[y]

SW, seawater; KX, konjac glucomannan–xanthan gum; LT FM, low temperature fish meal.
[1]Protein 888.52 g kg^{-1} of dry matter; carbohydrate 61.35 g kg^{-1} of dry matter; fat 6.85 g kg^{-1} of dry matter; ash 43.28 g kg^{-1} of dry matter.
[2]Protein 125.56 g kg^{-1} of dry matter; carbohydrate 524.13 g kg^{-1} of dry matter; fat 5.6 g kg^{-1} of dry matter; ash 344.71 g kg^{-1} of dry matter.
[3]Protein 187.7 g kg^{-1} of dry matter; carbohydrate 569.34 g kg^{-1} of dry matter; fat 6.26 g kg^{-1} of dry matter; ash 236.70 g kg^{-1} of dry matter.
[4]Protein 73.55 g kg^{-1} of dry matter; carbohydrate 562.02 g kg^{-1} of dry matter; fat 19.76 g kg^{-1} of dry matter; ash 344.68 g kg^{-1} of dry matter.
[5]Protein 731.71 g kg^{-1} of dry matter; carbohydrate 19.09 g kg^{-1} of dry matter; fat 89.08 g kg^{-1} of dry matter; ash 160.13 g kg^{-1} of dry matter.
[a]Vitamin premix composition per kg of experimental feed (Mai et al., 1995b): thiamine HCl 120 mg; riboflavin 100 mg; folic acid 30 mg; PABA 400 mg; pyridoxine HCl 40 mg; niacin 800 mg; Ca pantothenate 200 mg; inositol 4000 mg; ascorbic acid 4000 mg; biotin 12 mg; vitamin E 450 mg; menadione 80 mg; vitamin B_{12} 0.18 mg; vitamin A 100,000 IU; vitamin D 2000 IU; ethoxyquin 400 mg.
[b]Mineral premix per kg of experimental feed (Mai et al., 1995b; Tan et al., 2001): NaCl 0.4 g; $MgSO_4 \cdot 7H_2O$ 6.0 g; $NaH_2PO_4 \cdot 2H_2O$ 10.0 g; $KH_2PO_4 \cdot 20$ g; $Ca(H_2PO_4)_2 \cdot H_2O$ 8.0 g; Ferric citrate 1.0 g; $ZnSO_4 \cdot 7H_2O$ 141.2 mg; $MnSO_4 \cdot 2H_2O$ 64.8 mg; $CuSO_4 \cdot 5H_2O$ 12.4 mg; $CoCl_2 \cdot 6H_2O$ 0.4 mg; KIO_3 1.2 mg; Na_2SeO_3 0.4 mg.
[c]Values given as g kg^{-1} of dry matter.
[d]Energy values in kJ g^{-1}.
[x,y]Letters indicate the results of the Tukey HSD. Values in the same row that do share common superscript parameters were significantly different from each other ($P < 0.05$). Experimental parameters over the duration of the dry matter leaching experiment were: temperature (°C) = 11.25 ± 0.302; pH = 8.19 ± 0.006; O_2 (mg L^{-1}) = 9.74 ± 0.132.

energy value of the feeds was calculated from the proximate composition using the equation (A.O.A.C., 1990):

$$Energy(kJ/g) = [4(Protein) + 9(Lipid) + 4(Carbohydrate)] * 4.186/100.$$

Proximate composition and energy composition of the feed treatments are shown in Table 1.

2.2. Experimental parameters

Seawater was monitored weekly using a Palintest® photometer to measure levels of ammonia (0.43 ± 0.087 mg L^{-1} N), nitrite (0.18 ± 0.162 mg L^{-1} N), nitrate (0.79 ± 0.045 mg L^{-1} N), pH (8.23 ± 0.004) and alkalinity (190.73 ± 16.59 mg L^{-1}). Salinity averaged at 35.8 ± 0.100 g L^{-1} over the duration of the study. Seawater temperature and dissolved oxygen averaged at 14.59 ± 0.527 ºC and approximately 9.7 ± 0.152 mg L^{-1}, respectively, over the duration of the study.

2.3. Experimental design

Hatchery produced *H. discus hannai* [20–30 mm shell length], which were reared on *L. digitata*, were collected from Brandon Bay Seafoods, County Kerry Ireland in August 2008 and transported to the Aquaculture and Fisheries Development Centre, University College Cork (AFDC, UCC) in large, cable-tied plastic bags that were dampened internally with seawater and pumped with oxygen to reduce gill desiccation and aerobic stress (Sales and Britz, 2001). Abalone were maintained on a ration of *L. digitata* at the AFDC for 25 days to acclimate to the change in conditions. After the 25-day acclimation period, the *H. discus hannai* were starved for 7 days prior to the initiation of the experiment. Individuals were drained of excess surface moisture prior to measuring for individual shell length (±0.1 mm) and weight (±0.01 g) and randomly divided between 18 experimental tanks ($n = 15$ $tank^{-1}$). The initial stocking parameters of each tank were 24.53 ± 0.140 mm shell length and 2.28 ± 0.041 g live weight (mean ± S.E.).

This study was conducted over 12 weeks and consisted of six experimental treatments of which 5 feeds were novel formulated feeds. The control treatment was fed locally harvested *L. digitata*. Kelp was chosen as the experimental control because this is the predominant feed type currently used in commercial abalone culture in Ireland. Three replicates of each feed treatment were conducted. The experimental set-up allowed interchanges of tank position at each feeding interval for a completely randomised experimental design.

2.4. Feeding, consumption and growth

Feed replenishment was conducted every three to four days and abalone were fed ad libitum. Dry matter conversion factors for the artificial feeds being fed throughout the trial were obtained by drying 5 random samples of each diet in the oven at 105 ºC overnight at the beginning of the experiment. Fresh *L. digitata* was obtained from Ballycotton Bay, Co. Cork, approximately every 7–10 days, and stored in an aerated seawater tank at the AFDC. The *L. digitata* was removed from the seawater as required and all epiphytes were removed. At each feeding interval, a sample of the *L. digitata* frond used in feeding was dried in an oven ($n = 3$) at 105 ºC for 24 h to obtain a dry matter conversion

factor for that feed. All conversion factors for day 0 feeds were calculated as follows:

$$C = d/w$$

where C = conversion factor, d = dry weight of the feed (g) and w = wet weight of the feed (g).

Control samples (triplicate) of fresh *L. digitata* were run at each feeding interval to correct for loss in macroalgal biomass in the absence of abalone. Correction factors for KX feed consumption were determined from a seawater stability test conducted at the end of the experiment.

Daily feed consumption rates (DFC) and food conversion efficiencies (FCE) were calculated using the formula:

$$DFC = \left[\left(F_g - F_u\right)/t\right]/(W)$$

where the DFC was the daily food consumption (mg $_{\text{dry matter}}\, g^{-1}{}_{\text{abalone}}$ day^{-1}), F_g was the dry weight (g) of food given during the experimental period, F_u was the dry weight (g) of food uneaten during the experimental period, W was the mean wet weight of abalone during the experimental period (assuming linear growth) and t was the time in days. FCE was calculated from the formula:

$$FCE = 100\left(W_f - W_i\right)/\left(F_g - F_u\right)$$

where FCE was the food conversion efficiency (%), W_f and W_i were the final and initial whole abalone wet weights (g), F_g was the dry weight of food given (g) during the experimental period, and F_u was the dry weight of food uneaten (g) during the experimental period.

PER was calculated from the formula:

$$PER = W_f - W_i/P_g$$

where PER was the protein efficiency ratio ($g_{\text{weight gain}}\, g^{-1}{}_{\text{protein intake}}$), W_f and W_i were the final and initial whole abalone wet weights (g), and P_g was the dry weight of protein consumed (g) during the experimental period.

Initial and final biometric data (length; mm and weight; g) were recorded during this study to minimise the impact of sampling intervals on overall *H. discus hannai* health and homeostasis (Sales and Britz, 2001). Growth rate was calculated in terms of (i) shell length (linear growth rate; LGR) and (ii) total weight (specific growth rate; SGR) using the following equations:

$$LGR\left(\text{mm shell length day}^{-1}\right) = \left(L_f - L_i\right)/t$$

$$SGR\left(\%\text{day}^{-1}\right) = 100\left(\ln W_f - \ln W_i\right)/t$$

where L_i and W_i are initial length and weight, respectively, L_f and W_f are the final length and weight, respectively, t is time (days) and ln is the natural log.

The body weight to shell length ratio (BW/SL) was calculated at the beginning of this study and for all experimental treatments at the end of the study using the equation:

$$BW : SL\left(g\ mm^{-1}\right) = \text{Mean weight/mean length.}$$

Each tank was checked for mortalities at each feeding interval. Overall percentage survival (St) was calculated as the ratio of the number of individuals surviving at the end of experiment to the number of individuals at the beginning of the experiment using the equation:

$$St = N_t/N_o \times 100$$

where N_t is the number of abalone surviving at the end of the experiment and N_o is number of abalone at the beginning of the experiment.

2.5. Dry matter leaching

Experiments to determine the percentage dry matter loss of the feed treatments in this study in the absence of abalone were conducted over three to four days at the end of this study in the experimental system vessels. The dry matter loss values of the formulated KX feeds were used for corrected consumption estimates in this study.

The aquaria were labelled with the diet treatments of this study (n = 3). Into each tank, two pieces of the appropriate feed were placed. Each feed piece was pre-weighed and labelled with a twine tag corresponding to the days of the stability test (1st and 2nd). On day 0 of the dry matter leaching experiment, dry matter conversion factors for the feeds in the stability test were obtained using identical procedures to those outlined earlier (see Section 2.4). These values were used to calculate the average dry matter (g) in each feed piece used in the seawater stability test. On each day, the feed piece with the label corresponding to that day number (1 to 2) was removed from the aquaria, drained on absorbent paper, weighed and oven-dried at 105 °C for 24 h to determine the dry weight (g) of the feed piece. The percentage dry matter loss on each day was calculated using the equation:

$$\text{Dry matter loss}\left(g\ kg^{-1}\right) = (D_o - D_m)/D_o \times 10^3$$

where D_o is the dry matter (g) in the original feed piece, and D_m is the dry matter (g) in the final feed piece after immersion in seawater.

2.6. Statistical analyses

Tank position was randomised throughout the experiment and the possibility of tank effects was assessed using a randomised ANOVA at the end of the experiment. The assumptions of normality were analysed using the Kolmogorov–Smirnov or Shapiro–Wilk's test. Comparisons between groups of normal data were tested using ANOVA. Non-normal data were compared using the non-parametric Kruskal–Wallis test. Post-hoc analyses were conducted using the Tukey multiple comparison test and the Kruskal–Wallis multiple comparison test. All statistical analyses were conducted using SPSS (version 20). Significance was assumed when $P < 0.05$.

3. Results

3.1. Initial census and tank effect

A one-way ANOVA indicated that there was no significant difference in the mean weight of each tank at the outset of this study (ANOVA; $d.f. = 17, 269$, $F = 0.115$; $P = 1.000$).

The possibility of tank effect was tested at the end of the experiment by randomised complete block ANOVA with tank as a factor. Tank effect was non-significant ($P > 0.05$).

3.2. Dry matter leaching of experimental feeds in seawater

Immersion in seawater resulted in dry matter loss in all experimental treatments (Table 1).

Significant differences in the cumulative dry matter loss between treatments were observed on day 3 (ANOVA; $d.f. = 5, 17$; $F = 12.030$; $P = 0.000$) and on day 4 (ANOVA; $d.f. = 5, 17$ $F = 7.280$; $P = 0.002$). Dry matter loss from *L. digitata* was significantly lower than all experimental diet treatments. There was no significant difference in dry matter loss between the experimental feed treatments.

3.3. Abalone survival

At the end of the experiment, mean percent survival ranged from 88.89 ± 4.44% for Diet E treatment to 100 ± 0.00% for abalone fed *L. digitata* and Diet C (Table 2). There were no significant differences in the survival rates between treatments (Kruskal–Wallis; $d.f. = 5$; $H = 9.507$; $P = 0.090$).

3.4. Daily food consumption (DFC)

Mean DFC rates (mg DM g abalone^{-1} day^{-1}) ranged from 5.73 ± 0.41 mg (Diet E) to 13.06 ± 0.56 mg (Diet D). Significant differences in the DFC of *H. discus hannai* were observed (ANOVA; $d.f. = 5, 17$; $F = 34.723$; $P = 0.000$) (Table 2). The DFC of Diet C (5.99 ± 0.231 mg) and Diet E (5.73 ± 0.419) was significantly lower than that of fresh kelp (8.75 ± 0.645 mg), whereas the DFC of Diet D (13.06 ± 0.559 mg) was significantly higher than that of fresh kelp.

3.5. Food conversion efficiency (FCE)

Diet E had a significantly higher FCE (5.61 ± 0.546% weight gain per unit dry matter consumed) than *L. digitata* (3.22 ± 0.029%), Diet A (2.07 ± 0.292%), Diet B (2.32 ± 0.047%) and Diet D (2.42 ± 0.099%) (ANOVA; $d.f. = 5, 17$; $F = 8.833$; $P = 0.001$) (Table 2). There was no significant difference between the FCEs of any remaining treatment combination in this study.

3.6. Protein efficiency ratio (PER)

There was a significant difference in the protein efficiency ratio (PER) between *L. digitata* (2.58 ± 0.157 g $_{\text{weight gain}}$ g^{-1} $_{\text{protein intake}}$) and Diet D (3.26 ± 0.031 g $_{\text{weight gain}}$ g^{-1} $_{\text{protein intake}}$) (ANOVA; $d.f. = 5, 17$; $F = 4.265$; $P = 0.018$) (Table 2). There were no significant differences between the PER values of all remaining dietary treatments.

3.7. Growth

3.7.1. Linear growth rate (LGR)

Significant differences in the linear growth rate (LGR) between the *L. digitata* treatment (0.049 ± 0.004 mm day^{-1}) and Diet B (0.032 ± 0.003 mm day^{-1}) were evident (ANOVA; $d.f. = 5, 17$; $F = 4.676$; $P = 0.013$) (Table 2). No significant differences were observed between all remaining combinations of dietary treatments in this study.

3.7.2. Specific growth rate (SGR)

Significant differences in the specific growth rate (SGR) between treatments were observed (ANOVA; $d.f. = 5, 17$; $F = 3.819$; $P = 0.027$) (Table 2). The highest specific growth rate (SGR) in this study was observed in the Diet D treatment which had a mean SGR of 0.48 ± 0.015% day^{-1}. This treatment had a significantly higher SGR than Diet A (0.31 ± 0.045% day^{-1}) which had the lowest SGR of all treatments. No significant differences were observed between all other experimental treatments.

3.8. Body weight/shell length ratios (BW/SL)

At the end of this study, all dietary treatments demonstrated significant increases in BW/SL ratios when compared with the initial BW/SL ratio at the outset of this study (ANOVA; $d.f. = 6, 20$; $F = 11.399$; $P = 0.000$). There was no significant difference in the BW/SL ratio between experimental treatments on conclusion of this study (Table 2).

4. Discussion

4.1. Dry matter leaching of experimental diets

The dry matter leaching experiment indicated that *L. digitata* was a more seawater-stable feed than all novel artificial feed compositions with significantly less percentage dry matter loss on day 3 and day 4. However, it was interesting that there was a 100% increase in the weight loss of *L. digitata* in the 24 hour period between day 3 and day 4 which indicated that even the fresh diet was deteriorating. No significant differences in the dry matter leaching between the novel diet formulations were observed. However, trends indicated that Diet A (abalone base) had the lowest percentage dry matter loss than all other formulations which may have potentially occurred as a result of a greater degree of particle size homogeneity in the diet mix for Diet A. The significance of feed particle size on dry matter leaching has been previously discussed in O'Mahoney et al. (2011). A greater degree of feed particle homogeneity <450 μm can reduce dry matter leaching from prepared feeds (Sales and Britz, 2002) whilst a feed particle homogenate of 124 μm may improve overall feed performance (Obaldo et al., 1999). With 25–30% dry matter loss from the KX feeds, further research would need to be conducted to determine the optimum feed particle size for the KX feeds as the feed development programme advances.

4.2. The effect of diet type on DFC, FCE, PER, LGR, SGR, BW/SL ratio and survival

Daily feed consumption (DFC) in this study indicated that the use of a mixed seaweed meal in the novel feed formulations Diet C and Diet E resulted in significantly lower observed DFC values. Other basal dietary treatments in this study, including Diet A, Diet B and *L. digitata* showed no significant differences in DFC between these feed treatments. Given that the DFC of Diet D was significantly higher than all other feed treatments it is perceived that the combination of fishmeal and *L. digitata* meal as the major feed components may have functioned as chemo-attractants to increase ingestion. However, further investigation into the chemo-attractant properties of both fishmeal and *L. digitata* meal in formulated feeds for *H. discus hannai* is necessary before their role in increased ingestion rates can be determined.

This study demonstrated a negative correlation between consumption rates (DFC) and food conversion efficiencies (FCE) for *H. discus*

Table 2
Consumption and growth parameters of *H. discus hannai* in the current experimental study (mean ± S.E.; $n = 3$).

Diet	DFC (mg $_{\text{dry matter}}$ g^{-1} $_{\text{abalone}}$ day^{-1})	FCE (%)	PER (g weight gain per g protein intake)	LGR (mm day^{-1})	SGR (% day^{-1})	BW: SL (g mm^{-1})	Survival (%)
Initial data	–	–	–	–	–	0.093 ± 0.0005^{a}	
Laminaria digitata	8.75 ± 0.645^{a}	3.22 ± 0.023^{a}	2.58 ± 0.157^{a}	0.05 ± 0.004^{a}	0.43 ± 0.028^{a,b,c}	0.115 ± 0.002^{b}	100 ± 0.00
Diet A	9.89 ± 0.396^{a}	2.07 ± 0.292^{a}	2.83 ± 0.102^{a,b}	0.04 ± 0.003^{a,b}	0.31 ± 0.045^{b}	0.109 ± 0.002^{b}	97.78 ± 2.22
Diet B	10.57 ± 0.501^{a}	2.32 ± 0.347^{a}	2.84 ± 0.156^{a,b}	0.03 ± 0.003^{b}	0.36 ± 0.052^{a,b,c}	0.111 ± 0.005^{b}	93.33 ± 3.85
Diet C	5.99 ± 0.231^{b}	4.11 ± 0.861^{a,b}	2.99 ± 0.103^{a,b}	0.04 ± 0.003^{a,b}	0.43 ± 0.024^{a,b,c}	0.118 ± 0.003^{b}	100 ± 0.00
Diet D	13.06 ± 0.559^{c}	2.43 ± 0.099^{a}	3.26 ± 0.032^{b}	0.05 ± 0.002^{a,b}	0.48 ± 0.015^{c}	0.119 ± 0.001^{b}	97.78 ± 2.22
Diet E	5.73 ± 0.419^{b}	5.61 ± 0.546^{b}	3.02 ± 0.050^{a,b}	0.05 ± 0.001^{a,b}	0.46 ± 0.005^{a,b,c}	0.118 ± 0.003^{b}	88.89 ± 4.44

^{a,b,c}Superscript letters indicate the results of the Tukey HSD. For each column, values that do not share common superscript parameters were significantly different from each other ($P < 0.05$).

hannai fed the formulated diets. Although consumption was highest for abalone fed Diet A, Diet B and Diet D, these diets exhibited significantly lower FCEs than Diet E. Such a relationship has previously been described for *H. discus hannai* in this feed development programme (O'Mahoney et al., 2011) and also for the sea urchins *Paracentrotus lividus* and *Psammechinus miliaris* fed *Laminaria saccharina* (Cook and Kelly, 2007). However, a positive relationship between DFC and FCE was observed for *H. discus hannai* fed *L. digitata*.

Consumption in abalone is a difficult parameter to assess quantitatively and in a number of growth studies, consumption was not evaluated (see O'Mahoney et al., 2011 for a review of the literature). The importance of feed consumption in a novel feed development programme has been outlined in previous studies because the acceptability of the feed to the cultured animals can be discerned through consumption. However, for many commercially focused research programmes, FCE is the vital feed parameter to evaluate because it converts readily into final production costs for a producer whilst experimental growth rates may not extrapolate to a commercial setting. In this study, Diet E had a significantly higher FCE than fresh *L. digitata* and is therefore indicative of a feed formulation with development potential for *H. discus hannai*. Furthermore, Diet E was the first novel feed produced in a 3-year feed development programme to demonstrate higher feed efficiency in any feed formulation when compared with fresh *L. digitata* (O'Mahoney, 2009).

Although Diet D had a significantly different protein efficiency ratio (PER) to fresh *L. digitata*, the lack of a significant difference in the PER between the formulated feed diet treatments is an inference that overall diet performance was not a function of the protein content of the diets alone, although the net protein utilisation may have differed between the diets. A detailed study of the nutritional value of the protein sources and component diet ingredients for *H. discus hannai* is necessary before their role in overall diet performance can be elucidated.

Linear growth rates in this study fell within the range projected by Lee (2004) of 1–2 cm per annum for *H. discus hannai*. SGR in this study was lower than that of previous studies (O'Mahoney, 2009; O'Mahoney et al., 2011), possibly due to below target average seawater temperatures brought on by below average ambient air temperatures. The higher linear growth rates and specific growth rates observed in *L. digitata*, Diet C, Diet D and Diet E further indicated that the basal feed compositions of Diet A and Diet B were inadequate formulations for commercial scale *H. discus hannai* production. For LGR, *L. digitata* had the highest growth rate, approximately 0.002 mm shell length day^{-1} higher than Diet D. However, Diet D had the highest SGR of all experimental treatments and where growth in terms of shell length is not restricted, an artificial diet that induces higher weight gain in perhaps more favourable for commercial species culture. Although no significant differences were observed, trends indicated that three out of five novel feed formulations in this study (Diet C, Diet D & Diet E) produced better growth rates in terms of weight gain than the control treatment, *L. digitata*. This trend was reflected in the BW/SL ratios where the higher values for Diet C, Diet D and Diet E indicated that these feeds converted more readily into a commercially valuable product. It is likely that diet performance could be improved with some modification of the existing diet formulations.

In this study two experimental treatments achieved maximal survivorship, *L. digitata* and Diet C. Diet C was the first novel feed in this feed development programme to exhibit zero mortality (O'Mahoney, 2009). A single mortality event in which three individuals died in Diet E contributed to this treatment having the lowest percentage survival of all experimental treatments. High survival rates are typical in *H. discus hannai* culturing studies (O'Mahoney et al., 2011) and although Diet E was perceived have an overall low survival rate compared with the other diet treatments in this study, the lack of significance between treatments coupled with the evidence outlined in O'Mahoney et al. (2011) suggests that the observed survival rates were within published ranges for the species, e.g. 84–95% (Mercer et al., 1993), 86.7–96.7% (Mai, 1998), and 88–100% (Mai and Tan, 2000).

The addition of lipids and choline chloride to the basal abalone feed (Diet B) increased DFC of this feed over the basal feed but not significantly so. The FCE of Diet A and Diet B was significantly lower than that of *L. digitata* and it is unlikely that these diets are acceptable for commercial *H. discus hannai* production given that FCE is of particular importance to commercial producers. The lipid concentrations in this study were within the target range for this species of abalone (Mai et al., 1995b). Although consumption was not quantified in their study, Mai et al. (1995b) observed lower consumption in feeds containing high lipid levels (11.58%) for *H. discus hannai* and *Haliotis tuberculata*. Lower feed intake at high lipid levels was observed by Thongrod et al. (2003) and was proposed to contribute to poorer growth of donkey's ear abalone, *Haliotis asinina*.

The digestibility of certain lipids to abalone has been questioned. An artificial feed containing fish oil induced significantly lower lipase activity in juvenile black abalone (*Haliotis rubra*) (Johnston et al., 2005) and low lipase activity has been found in the gut of *H. midae* (Knauer et al., 1996). The apparently low lipid requirements of abalone appears to be an adaptive response to a natural food type that is low in lipids and high in carbohydrates (Durazo-Beltran et al., 2004). Although lipids are an important component of abalone tissue, particularly the gonad (Mai et al., 1995b) a number of authors suggest that protein and carbohydrate are more important than lipids for energy (Durazo-Beltran et al., 2004; Fleming et al., 1996; Thongrod et al., 2003). It has been suggested that starvation periods exceeding 70 days are necessary before lipid reserves are utilised for energy in *Haliotis fulgens* (Durazo-Beltran et al., 2004). Mai et al. (1995b) have suggested that a strategic approached to feed formulation could ensure that the essential fatty acid requirements of abalone are satisfied without specific dietary lipid supplementation. Indeed, the comparatively higher lipid concentrations of the diets containing fishmeal in this study (Diet D and Diet E) appear to indicate that the natural lipid concentration of fishmeal may be utilised to formulate a feed with the target lipid concentration and essential fatty acid composition. However, the assumption that the essential fatty acid composition of fish meal was sufficient for abalone cultivation without further dietary lipid supplementation will need further investigation for the KX feeds. However, it is worth noting that a number of feed formulations outlined in Fleming et al. (1996) utilise the bound lipids in fishmeal as the only lipid supply in the feed formulations.

The inclusion of mixed seaweed meals in the formulation of Diet E resulted in a significantly higher FCE than all other feed treatments that did not contain mixed seaweed meals in the formulation. Although no significant differences were observed, trends showed that the growth parameters measured in this study indicated that Diet C, containing mixed seaweed meals, performed better than the basal formulation counterparts (Diet A and Diet B). Coupled with this, the maximal survivorship supported by Diet C is very favourable for commercial *H. discus hannai* production. This study has shown that the use of *L. digitata* meal, *P. palmata* meal and *U. lactuca* meal in equal proportions is of notable value in the novel artificial feed formulation with 28.5% increased linear growth rates, 20% increase weight gain and 6.66% higher survival rates when compared with Diet B which was similar in formulation with only *L. digitata* meal accounting for the entire seaweed meal proportion of this feed. A recent report indicated that 5% dietary supplementation with red algae in a natural seaweed diet can increase growth rates of red abalone by 25% (Frazer, 2009) but the current study has highlighted the prospects for a mixed seaweed meal formulated feed for *H. discus hannai*.

U. lactuca and *P. palmata* have been identified as suitable algae for abalone culture (Mercer et al., 1993; Uki et al., 1986b) but alone can produce poor growth rates when supplied as the only feed option (Fleming, 1995; Mercer et al., 1993). However, when cultured under enrichment conditions the performance of these algae on abalone growth when

presented as the only feed item may be improved considerably (Neori et al., 1998; Shpigel et al., 1999). Similarly, three species of red algae (*Hynea spinella*, *Hynea musciformis* and *Gracilaria cornea*) have been identified as a suitable feed for *Haliotis tuberculata coccinea* when cultured under enrichment conditions also (Viera et al., 2005).

The nutritional value of a mixed seaweed diet has been supported for abalone culture with red and green algae often preferred over kelp species (Day and Cook, 1995). A mixed fresh seaweed diet containing *E. maxima* and enriched *G. gracilis* and *U. lactuca* produced better growth in *H. midae* than a number of other feed treatments including fresh kelp (*E. maxima*), Abfeed®, kelp + Abfeed®, various forms of dried kelp, fresh kelp with the epiphyte *Carpoblepharis flaccida* and a rotational diet of a mixture of these treatments (Naidoo et al., 2006). There is also some evidence to suggest that the polysaccharides contained within marine macroalgae may act as potential prebiotics (O'Sullivan et al., 2010) and are, therefore, of some utility to enhance growth and condition in aquaculture. It is possible that the use of enriched seaweed meals with high protein concentrations originating principally from farm origin ammonia-N (Sanderson, 2006) may be particularly suitable for artificial feed formulation and further investigation of this research topic is highly recommended. Indeed, salmon farming is well established in Ireland. The effective production of enriched seaweeds adjacent to existing salmon farms has been demonstrated by Sanderson et al. (2008), so the potential exists for a spin-off industry to be established for seaweed culture with an existing market for artificial feed development.

This study also demonstrated conflicting results from the use of fishmeal in the novel feed formulation. Diet D performed better than all other novel diets in terms of linear and specific growth rates but the high consumption rates for this feed attributed to a low food conversion efficiency rating and ultimately higher costs for commercial production. The improved growth performance from Diet B formulation to Diet C was not reflected from Diet D to Diet E but overall Diet E was more efficient that Diet D. The feeds containing fishmeal (Diet D and Diet E) had a lower concentration of seaweed meal than other formulations (Diet A, Diet B & Diet C) which contributed to a lower percentage carbohydrate than the diets containing fish meal. However, the carbohydrate concentrations in these feeds did not appear to be a critical parameter in the comparative feed performance of the formulated feeds because both linear and specific growth rates in both Diet D and Diet E exceeded other formulations. The most notable difference between Diet D and Diet E was in the percentage survivorship and the reason for this difference is unknown. Fleming et al. (1996) indicated the diets containing >20% fishmeal can be detrimental to abalone due to excessive phosphorous. Based on evidence in Sales et al. (2003), the target fishmeal concentration for the fishmeal-containing feeds in this study was 20%, a level which falls approximately mid-way between the fishmeal inclusion range of a number of artificial feeds (Fleming et al., 1996). It is not known if the mixed seaweed meal contributed to greater phosphorous in these feeds, although the uptake of this nutrient has been demonstrated for marine algae (Kitadai and Kadowaki, 2007; Lobban and Harrison, 1994). Without a complete profile of the feeds and the individual abalone feeding on them this perplexing result will not be resolved.

4.3. Conclusions and further research

This study demonstrated that the novel KX binder is suitable for the development of prepared feeds for *H. discus hannai* culture. Moreover, this study has demonstrated that enhanced diet performance can be attained with a comprehensive diet formulation but adequate control of the final diet composition would require further modification to the diet formulation sheet.

Assuming linear growth, the linear growth rates in this study approximated to the published annual growth for *H. discus hannai* but specific growth was lower than published ranges. When taken in their entirety, the result of this study supports further investigation into the use of these novel KX binders as a feed production alternative for artificial feeds for commercial abalone culture and there would be some benefit to derive from validation of the formulated diets in a commercial setting with established growth rates. In particular, further diet development to reduce the dry matter leaching of the experimental KX feeds, with a possible evaluation of the influence of ingredient particle size on overall KX feed performance, would be worthwhile.

Trends indicated that the best performing diets in this study were Diet C, Diet D and Diet E. The lack of a significant difference between Diet C and Diet D or Diet E in terms of percentage survival, FCE, LGR, SGR and BW:SL ratio indicated that the use of a mixed seaweed meal containing *L. digitata* meal and low temperature processed *P. palmata* meal and *U. lactuca* meal may be favourable as an alternative to low temperature fishmeal in formulated feeds for *H. discus hannai*, although some diet formulation refinement may be necessary to achieve growth rates of interest to industry. In commercial settings where fishmeal is a licensed commodity and unsustainable resource, high performance plant-based feeds would be very favourable. Further study investigating the suitability of enriched seaweed meals for use in abalone feeds is recommended. It is also suggested that further investigation is conducted to determine the cause of reduced growth and percentage survival in Diet E when low temperature fishmeal and the mixed seaweed meal ingredient components were combined in a single diet formulation.

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