ELEMENTA
Science of the Anthropocene

Andrade, S, et al. 2024. Enzyme-treated microalgal co-product diets for rainbow trout aquaculture: Supporting fish growth, phosphorus digestibility, and reducing phosphorus waste emission. *Elem Sci Anth*, 12: 1. DOI: https://doi.org/10.1525/elementa.2023.00119

RESEARCH ARTICLE

Enzyme-treated microalgal co-product diets for rainbow trout aquaculture: Supporting fish growth, phosphorus digestibility, and reducing phosphorus waste emission

Sofie Andrade[1,†], Pallab K. Sarker[1,†,*], Anne R. Kapuscinski[1], Devin Fitzgerald[1], Connor Greenwood[2], Pablo Nocera[1], Kira O'Shelski[1], Benjamin Lee[1], Abel Mkulama[1], Duncan Gwynne[1], Diego Gonzalez Orcajo[1], Benjamin Schoffstall[1], Uchasha Sarker[3], and Lydia Warkaw[1]

Aquaculture is one of the fastest-growing food sectors, producing almost 50% of fish for human consumption worldwide. However, relying on fish meal and oil for aquaculture diets is not sustainable economically or environmentally. Aquaculture feeds also contain terrestrial plant ingredients with indigestible forms of phosphorus (P), of which 70%–80% can be released into aquatic environments. This P influx contributes to eutrophication of freshwater ecosystems that can lead to anoxic conditions. This study explores a more sustainable diet for salmonids, an important and valuable seafood. Our aim was to test ingredients with highly digestible forms of P in nutritionally balanced portions to support fish growth and reduce P loading. We determined the digestibility of three feeds containing raw, extruded, and enzymatically processed microalgal co-product of *Nannochloropsis oculata* compared to a conventional diet. We also quantified how much P was retained and excreted. We detected highest growth in trout fed enzymatically processed co-product feed, though it was not statistically different ($p = 0.846$) from growth of fish fed the reference or other co-product diets. The enzyme-treated, microalgal co-product ingredient and diet had comparable values for P digestibility and solid P excretion to the reference diet, but the lowest average solid P excretion of all test diets. Trout fed the enzyme-treated diet had the highest P retention, while the reference diet had the lowest ($p = 0.0429$). Trout fed the enzyme-treated diet had the lowest ($p = 0.0174$) and negative dissolved P excretion, while those fed the reference diet had the highest. Results showed that enzyme-treated *N. oculata* co-product maintains digestibility, increases P retention, and reduces dissolved P excretion compared to the reference diet in rainbow trout. These findings encourage follow-up research to design and test growth performance of diets containing enzyme-treated microalgal co-product as sustainable trout aquafeed.

Keywords: Marine microalgae, *Nannochloropsis oculata*, Co-product, Enzyme, Phosphorus, Digestibility, Loading, Salmonid, Rainbow trout

Introduction

Commercial capture fisheries are expected to remain at high levels through 2030, while aquaculture is expected to expand to 59% of human fish consumption in 2030 compared to 52% in 2018 (Food and Agriculture Organization [FAO], 2020). Within aquaculture, salmon and trout production is predicted to continue to grow through 2030; however, predicted price increases of 30% for fish meal and 13% for fish oil ingredients used in salmon and trout aquafeeds are expected to slow their growth (FAO, 2020). Growing demand for fish oil and fish meal inclusion in aquafeeds is concerning for several reasons. Sourcing fish meal and fish oil from forage fish is not economically sustainable due to increasing and more volatile costs, given that about 75% of current annual fish oil production goes into aquafeeds. Sourcing from forage fish undermines environmental sustainability by depleting forage fish from marine food webs that support seabirds, marine mammals, and predator fish. It also diverts small pelagic fish of high nutritional quality away from human consumption into reduction fisheries; for example, sardines offer high nutritional benefits via long-chain

[1] Environmental Studies Department, University of California Santa Cruz, Santa Cruz, CA, USA
[2] Monterey Bay Salmon and Trout Project, Davenport, CA, USA
[3] Santa Cruz High School, Santa Cruz, CA, USA
[†] Contributed equally
* Corresponding author:
Email: psarker@ucsc.edu

polyunsaturated fatty acids (PUFAs) in their oil (FAO, 2020), as well as high calcium and lean protein content for human consumption.

To reduce their production costs, feed manufacturers have begun modifying their feed formulas to replace fish meal and oil with plant or animal ingredients that have lower digestibilities (Bureau and Hua, 2010). But most waste released by aquaculture systems is due to poor digestibility of fish diet ingredients that are then released through solid waste (Bureau and Hua, 2010; Suresh et al., 2023). Phosphorus (P), in particular, is a concerning waste because of its role as a limiting factor for algal growth in freshwater environments (Bureau and Hua, 2010). For example, fishmeal has high levels of ash due to high bone content, containing a large percentage of P, mainly as hydroxyapatite; however, 86%–88% of this P is inaccessible to fish (Sugiura et al., 1999), leading to environmental P loadings (Bureau and Hua, 2010; Sarker et al., 2011). The nutritional disadvantages of terrestrial plant ingredients limit their inclusion rates in diets due to high phytate-bound P which is largely indigestible to monogastric animals like fish (National Research Council [NRC], 1993; Mainstone and Parr, 2002; Sarker et al., 2013). An increase in P emissions into the nutrient cycles of an aquatic ecosystem can lead to eutrophication, the excessive growth of primary producers (algae), and anoxic conditions (Bureau and Hua, 2010; Sugiura, 2018; Gamble et al., 2021; Suresh et al., 2023). Additional knowledge surrounding the composition and bioavailability of certain nutrients in feed ingredients is needed in order to formulate a nutritionally complete feed that also reduces harmful ecological impacts associated with excess nutrients in aquaculture effluents.

To provide a nutritionally balanced diet for salmonids, researchers are exploring the use of marine microalgae as a substitute for traditional sources of ingredients (Sørensen et al., 2017; Gong et al., 2018; Sarker et al., 2018). Recent research has shown the potential for using *Nannochloropsis* spp. defatted co-products, notably for Atlantic salmon. To the best of our knowledge, no research has comprehensively evaluated the incorporation of *N. oculata* co-product as a protein source for rainbow trout species. The literature lacks research conducted using *N. oculata* co-product for rainbow trout or other carnivorous fish species. It has been found to be a digestible source of protein and eicosapentaenoic acid for Atlantic salmon and could be used as a protein ingredient in aquafeed formulations for Atlantic salmon (Kiron et al., 2012; Sørensen et al., 2017; Gong et al., 2018). Nutritional feeding experiments using *N. oceania* biomass have been conducted with rainbow trout and Atlantic salmon and have shown that up to 10% of that biomass can be included in the feed without adverse effects on growth (Sørensen et al., 2017). Research is also lacking on the digestibility of P and nutrient emissions in microalgal trout feeds. Examining the P digestibility of microalgal ingredients is crucial for reformulating a more sustainable fish diet. Microalgae-supplemented diets have been found to have higher apparent digestibility coefficients (ADCs) for P in Nile tilapia (*Oreochromis niloticus*; Gamble et al., 2021). This previous study considered microalgae such as *Chlorella* sp., *Spirulina* sp., and *Schizochytrium* sp. and found that the latter had the highest ADC. These results support additional efforts for replacing fish meal and fish oil with microalgae as a sustainable alternative to reduce waste output from aquaculture systems.

The digestibility and retention of P in fish diets determine how much is excreted into aquatic environments (Bureau and Hua, 2010). Reformulating fish diets to contain highly digestible nutrient sources is a promising way to decrease levels of P excreted into aquatic ecosystems (Burea and Hua, 2010). However, using terrestrial crops and microalgae in rainbow trout diets can lead to the presence of anti-nutritional substances like non-starch polysaccharides (NSP), which remain undigested in monogastric animals like rainbow trout (Sarker et al., 2018; Maas et al., 2019). NSP are one main component of ingredients that form the rigid structure of plant and microalgal cells. In monogastric animals such as rainbow trout, NSP remain undigested as the animals lack the necessary enzymes, ß-glucanase and xylanases, to break them down (Scholz et al., 2014; Maas et al., 2019). After extracting lipids from *Nannochloropsis oculata*, the remaining co-product increases anti-nutrient levels, including NSP, in the *N. oculata* biomass. This increase results in lower fish growth when fed raw co-product diet (Sarker et al., 2018). Previous studies have suggested the need to evaluate if enzymatic and extrusion processing can enhance the nutritive quality of microalgal ingredients (Tibbetts et al., 2017; Sarker et al., 2018; Sarker et al., 2020). Combining exogenous enzymes such as protease, xylanase, and ß-glucanase can help break down anti-nutritional substances and reduce the negative impact of NSP on the digestibility and growth performance of fish (Choct, 1997; Maas et al., 2019).

This study aimed to find an effective way to enhance the digestibility of P in *Nannochloropsis oculata* co-product to help reduce P discharge from trout aquaculture. The study involved processing *N. oculata* co-product through extrusion and enzymatic processing. We then evaluated the P digestibility and P emissions in rainbow trout of raw *N. oculata* co-product, enzyme-treated and extrusion-processed co-product, and of test diets to assess feasibility of reducing environmental P emissions by using one or more of these forms in aquafeed for sustainable rainbow trout aquaculture.

Materials and methods

Dietary experimental design

A 30-day digestibility experiment compared the digestible nutritional values of three treatments of *Nannochloropsis oculata* co-product (raw, enzyme-treated and extruded at 90°C) versus fishmeal in a reference diet. We evaluated the ADCs of P in all test diets, including the reference diet. We followed the standard apparent digestibility protocol to prepare the three test diets (raw co-product, extruded co-product meal, enzyme-treated co-product; Cho et al., 1982; Sarker et al., 2016; Sarker et al., 2018; Sarker et al., 2020; Bélanger et al., 2021). First, we prepared a high quality, nutritionally complete reference diet

Table 1. Ingredient composition (g kg^{-1}) of the reference diet and the microalgal (*Nannochloropsis oculata*) co-product test diets for the digestibility experiment

	Diet			
Ingredient	**Reference**	**70% Ref + 30% Raw *N. oculata***	**70% Ref + 30% Enzyme-Treated *N. oculata***	**70% Ref + 30% Extruded *N. oculata***
Fish meal	300	210	210	210
Corn gluten meal	170	119	119	119
Wheat meal	174	122	122	122
Soybean meal	129	90.3	90.3	90.3
Wheat gluten	100	70	70	70
Vitamin/mineral premix	5	3.5	3.5	3.5
Fish oil	112	78.4	78.4	78.4
Yttrium oxide	10	7	7	7
N. oculata raw co-product	0	300	0	300
N. oculata enzyme treated co-product	0	0	300	0
N. oculata extruded co-product	0	0	0	300

(**Table 1**) and combined microalgal co-product biomass at a 7:3 ratio (as-is basis) to create the three test diets. We also included a digestion indicator in the basal diet at 0.1% consisting of the indigestible marker, yttrium oxide, from Thermo Scientific, Waltham MA, USA (Barrows et al., 2007). We mixed the micro-ingredients before slowly adding the macro-ingredients to ensure a homogeneous mixture. The thoroughly mixed ingredients were then steam-pelleted using a California Pellet Mill, dried in a forced-air oven (22°C, 24 h), sieved, and stored at −20°C. The total P, ADC of P (%), P retention, and P-waste outputs of the four test diets used in the digestibility experiment were determined as described in the following sections.

Methods of processing Nannochloropsis oculata *co-product*

Enzymatic processing

We used one enzymatic processing treatment containing a combination of xylanase, glucanase, and protease with previously determined appropriate doses for rainbow trout (Dalsgaard et al., 2016). Our previous experimental results informed the best dosage for the combination of enzymes as follows: xylanase (RONOZYME® WX L, DSM Nutritional Products, USA) at 208 mg kg^{-1}; glucanase (ß-glucanase, RONOZYME® VP (L), DSM Nutritional Products, USA) at 67 mg kg^{-1}; and protease (serine protease enzyme; DSM Nutritional Products, USA) at 228 mg kg^{-1} (Dalsgaard et al., 2016). We used this combination and dosage of enzymes to treat the *N. oculata* co-product.

Extrusion processing

We conducted extrusion-processing treatments of *N. oculata* co-product at the Kapuscinski-Sarker Lab space in Natural Sciences II (University of California, Santa Cruz) using a single-screw extruder (TT-100 tabletop lab scale extruder from Akron Tool and Die, Akron Ohio). The co-product was exposed to an average target temperature in the barrels of 90°C and passed through the extruder for an 18 s exposure. Extruded co-product was then ground to prepare it for use. During the extrusion process, the pressure varied at the die head with an average (± standard deviation, SD) of 1.04 ± 0.18 psi (n = 3), depending on diet moisture and screw speed. In addition, the screw speed was adjusted (according to the mash consistency) in order to achieve the appropriate mean (± SD) barrel retention time of 18.3 ± 0.17 s (n = 3). Higher moisture mash (>30%) had a motor speed of 38.3 rpm (± 3.5%) and lower moisture mash (<25%) had a speed of 45.4 rpm (± 6.5%).

Fish, feeding, and feces collection

Juvenile rainbow trout were randomly assigned to 100-gallon freshwater, rectangular tanks that made up recirculating aquaculture systems located at the University of California, Santa Cruz. There were 16 total tanks, and each tank had 16 fish with an average weight of 15.0 ± 2.5 g (192 trout total). The trout were then acclimated to the experimental conditions for 7 days and fed the reference diet. The water parameters were maintained within the limits recommended for rainbow trout. After the fish were acclimated, the digestibility trial began with each diet fed to 4 tank replicates. We hand-fed the fish twice a day between 0930 h and 1700 h and collected uneaten feed to prevent mixing with the fecal samples. Restricted pair feeding was applied to ensure that all groups received the same quantity of nutrients from the feed diets (Glencross et al., 2007; Sarker et al., 2016; Sarker et al., 2020). We carefully monitored water quality daily to maintain favorable conditions for rainbow trout across all tanks:

average water temperature 15.4°C, pH 8.6, dissolved oxygen 8.7 mg L^{-1}, total ammonia nitrogen 0.2 mg L^{-1}, nitrite nitrogen 0.1 mg L^{-1}, and nitrate nitrogen 26.8 mg L^{-1}. Fish fecal samples were collected from a fecal collection column attached to the bottom of each tank. They were collected before the morning and afternoon feedings for 30 days by the following process: closing a valve to seal off the fecal collection column, gently pipetting settled feces out of the column, and transferring the samples to a 50 mL Falcon tube (BD Falcon™; Sarker et al., 2016; Sarker et al., 2020). The fecal collection columns were flushed after each feeding to remove any uneaten feed or remnant feces. After the samples settled, we used the pipet to remove remnant water from the tubes and then froze the samples at −20°C. The fecal samples were grouped by tank throughout the entire experiment, after which we lyophilized, finely ground, and continued to store the samples at −20°C in preparation for further analysis.

Chemical analysis and calculations

Our previous studies informed the chemical analysis and digestibility calculations we performed for this study (Sarker et al., 2016; Sarker et al., 2018; Sarker et al., 2020). We analyzed three different types of samples, microalgal coproducts, diets, and feces, for proximate and P analysis.

The samples were assessed by performing acid digestions according to the EPA method 3050b for the acid digestion of sediment, sludges, and soils. The diet samples had 3 replicates and the fecal and whole fish body samples had 2 replicates. The fecal and whole fish body samples were grouped according to their tank of origin. Approximately 50 g of each sample were transferred into a separate beaker where acids were added, as per the EPA method 3050b instructions. Each sample was transferred to 10 mL HNO_3 (50%), 5 mL concentrated HNO_3, and 10 mL concentrated HCl, and heated over a hot plate to just before boiling to prevent evaporation of the solution. The prolonged process of heating the beakers broke down any solids from the samples and made the nutrients and elements available to analyze in the remaining solution. After completing each acid addition, the solution was heated until each beaker only contained 5 mL. After cooling, these solutions were treated one last time by filtering through filter paper (Whatman No. 41) into individually labeled bottles and increasing the volume to 50 mL by the addition of deionized water. The acid digestions were done in preparation for analysis by Inductively Coupled Plasma Optical Emission Spectroscopy to quantify the total amount of P (mg L^{-1}) present in the collected samples of diet, whole fish body, and feces.

We sent three samples (microalgal co-product, diets, and feces) to New Jersey Feed Laboratory, Inc. (Ewing, NJ, USA) for proximate analysis, and amino acid and fatty acid analysis. The analyses included moisture (method 930.15 of the Association of Official Analytical Chemists [AOAC], 1995), crude protein (AOAC 990.03), lipid (AOAC 920.39), ash (AOAC 942.05), crude fiber (AOAC 1978.10), energy (automated oxygen bomb calorimeter), amino acids (high-performance liquid chromatography analysis, via AOAC methods 994.12, 985.28, 988.15, and 994.12), fatty acids (fatty acid methyl ester analysis, via AOAC method 963.22.), and yttrium oxide in feed and feces according to methods described by Cho et al. (1982). We divided the crude protein results by a nitrogen conversion factor of 6.25 to quantify the amount of nitrogen in the diets, whole body fish, and feces.

Apparent digestibility coefficients were calculated for P and N of the test and the reference diets using the method described by Cho et al. (1982):

ADC = 100 × [1 − (% Nutrient of feces / % Nutrient of diet) × (% Marker in diet / % Marker in feces)]. For the ADC of the microalgae we used the equation proposed and simplified by Bureau and Hua (2006) and Jobling (2011):

$$\mathrm{ADC_{test\ ingredient}} = \mathrm{ADC_{test\ diet}} + [(\mathrm{ADC_{test\ diet}} - \mathrm{ADC_{ref\ diet}}) \times (0.7 \times \mathrm{D_{ref}}/0.3 \times \mathrm{D_{ingredient}})]$$

where D_{ref} is the percentage of nutrient (or kcal g^{-1} gross energy) in the reference diet, and $D_{ingredient}$ is the percentage of nutrient (or kcal g^{-1} gross energy) in the ingredient.

To calculate the percentage of digestible P and N nutrients (g kg^{-1}) of feed, the ADC was multiplied by the amount of nutrients in each diet. The P and N budget for each diet was calculated using methods from Cho et al. (1982), Sarker et al. (2014), and Gamble et al. (2021). To identify the nutrient (P and N) intake per gram of fish, we multiplied the feed per fish dry weight by the percentage of nutrient present in the dry feed as follows:

$$\text{Intake nutrient per fish (g)} = [(\text{Feed/Fish dry weight}) * (\%\ \text{Feed nutrient dry})]/100$$

To get the nutrient intake (g kg^{-1}), we took the intake nutrient (gram per fish) and divided it by the difference in final and initial dry weight of fish as follows:

$$\text{Intake of nutrient (g kg}^{-1}) = \frac{\text{Intake nutrient per fish (g)}}{\text{Final dry weight per fish (g)} - \text{Initial dry weight per fish (g)}} * 100$$

To calculate nutrient retention, we did the following:

$$\text{Retained nutrient per fish (g)} = [\text{Final dry weight per fish (g)} * \%\ \text{Final fish nutrient}\ /100] * [\text{Initial dry weight per fish (g)} * \%\ \text{Initial fish nutrient}\ /100]$$

followed by:

$$\text{Retained nutrient (g kg}^{-1}) = \frac{\text{Retained nutrient per fish (g)}}{\text{Final dry weight per fish (g)} - \text{Initial dry weight per fish (g)}} * 1000$$

To assess nutrient loading, we calculated the solid and dissolved nutrient waste from the fish. We calculated solid nutrient loading on feed (g kg^{-1}) using the equations:

$$\text{Solid nutrient per fish (g)} = \text{Intake nutrient per fish (g)} * [1 - (\%\ \text{Diet ADC of nutrient}\ /100)]$$

$$\text{Solid nutrient (g kg}^{-1}) = \frac{\text{Solid nutrient per fish (g)}}{\text{Final dry weight per fish (g)} - \text{Initial dry weight per fish (g)}} * 1000$$

Table 2. Growth indices (mean ± standard error, n = 4[a]) of rainbow trout fed the reference and experimental diets

	Diets[b]				
Index	**Reference**	**Raw**	**Enzyme-Treated**	**Extruded**	**p Value[c]**
Initial weight per fish (g)	26.7 ± 2.0 [a]	29.2 ± 2.0 [a]	29.2 ± 2.3 [a]	26.8 ± 2.0 [a]	0.7149
Final weight per fish (g)	46.5 ± 3.3 [a]	48.2 ± 3.3 [a]	50.2 ± 3.8 [a]	46.2 ± 3.3 [a]	0.8462
Weight gain[d] (g)	19.7 ± 1.30 [a]	19.0 ± 1.30 [a]	21.0 ± 1.50 [a]	19.4 ± 1.30 [a]	0.7883
FCR[e]	1.13 ± 0.03 [b]	1.33 ± 0.03 [a]	1.17 ± 0.03 [b]	1.15 ± 0.03 [b]	0.0011
SGR[f] (% day^{-1})	2.65 ± 0.04 [a]	2.40 ± 0.04 [b]	2.60 ± 0.05 [a]	2.58 ± 0.04 [ab]	0.0081

[a]The enzyme-treated diet was analyzed with 4 replicates (n = 4).

[b]Mean values not sharing a common bracketed letter in the same row differ significantly as determined by Tukey's HSD test, $p < 0.05$; both letters appearing together means no difference.

[c]These p values are for ANOVA comparing the mean values for each diet.

[d]Weight gain (g) = final wet weight (g) − initial wet weight (g).

[e]Feed conversion ratio (FCR) = feed intake (g) / weight gain (g), where weight gain (%) = [final wet weight (g) − initial wet weight (g) / initial wet weight (g)] × 100.

[f]Specific growth rate (SGR, % day^{-1}) = 100 × [ln final wet weight (g) − ln initial wet weight (g)] / time (days).

Finally, we used the solid nutrient (g kg^{-1}) to calculate the dissolved nutrient loading using the following equations:

$$\text{Rejected nutrient (g kg}^{-1}\text{)} = \text{Intake nutrient (g kg}^{-1}\text{)} - \text{Retained nutrient (g kg}^{-1}\text{)}$$

$$\text{Dissolved nutrient (g kg}^{-1}\text{)} = \text{Rejected nutrient (g kg}^{-1}\text{)} - \text{Solid nutrient (g kg}^{-1}\text{)}$$

Statistical analysis

We performed a one-way analysis of variance (ANOVA) of growth performance, ADC of P in diets and ingredients, amounts of P digested, and P budget fractions (retention, intake, total load, dissolved, and solid). Differences were considered statistically significant at $p < 0.05$. When significant differences were found, we conducted a Tukey's test of multiple comparisons to compare the means among the 4 treatments. Our results present the mean ± standard error (SE) of 4 replicates, excluding one of the enzyme-treated diet replicates. Tank R1 was the enzyme-treated diet replicate excluded from the analysis for P budget fractions as a statistical outlier due to the values lying outside of the 95% confidence interval.

Results

Growth indices of digestibility experiment

Table 2 summarizes the results of the 30-day growth data from digestibility experiment. The fish remained healthy and gained weight throughout the experiment. We found significant differences in specific growth rate (SGR, % d^{-1}; $F_{3,11} = 6.62$, $p = 0.0081$) and feed conversion ratio (FCR; $F_{3,11} = 11.31$, $p = 0.0011$) between experimental diets. However, we did not detect any differences in weight gain between treatments ($F_{3,11} = 0.35$, $p = 0.79$). The SGR was significantly higher in trout fed the reference diet (2.65) and enzyme diet (2.60) than in trout fed the raw diet (2.40). The FCR was significantly higher (poor) in trout fed the raw diet (1.33) than in those fed the reference (1.13), extruded (1.15), and enzyme diet (1.17).

Total P, digestibility, and digestible P content

The P content of the reference diet (37.02 g P kg^{-1} feed) was higher than the experimental diets (21.83–25.00 g P kg^{-1} feed; **Table 3**). The ADC of P in the enzyme diet did not differ significantly from the reference and raw co-product diets (63.01%; $F_{3,12} = 5.78$, $p = 0.01$). However, ADC of P in fish fed the reference (64.8%) and raw diets (63.6%) was higher than trout fed the extruded diet (57.3%). The digestible P (g kg^{-1}) significantly differed between diets, ranging from 13.76 (enzyme) to 24.01 (reference; $F_{3,12} = 231.8$, $p = 0.0001$). A Tukey HSD test showed that the amount of digestible P in the reference diet was significantly higher than all 3 microalgal co-product experimental diets. Among the test diets, the raw co-product diet had the highest digestible P but was only significantly greater than the enzyme diet.

The *Nannochloropsis oculata* raw co-product, extruded co-product, and enzyme-treated co-product contained 17 g P kg^{-1}, 17 g P kg^{-1}, and 19 g P kg^{-1} (total P content), respectively (**Table 4**). The ADC and amount of digestible P did not differ significantly among the experimental ingredients ($F_{2,9} = 3.61$, $p = 0.07$ and $F_{2,9} = 2.88$, $p = 0.11$, respectively).

P budget fractions

We report P budget fractions for trout fed different diets in **Table 5**. Fish fed these diets differed significantly in intake P ($F_{3,11} = 5.78$, $p = 0.013$), P retention ($F_{3,11} = 3.81$,

Table 3. Quantity and digestibility of phosphorus in the control (reference, n = 4) and three experimental diets (raw, enzyme-treated, and extruded; mean ± standard error, n = 4)

	Diets[a]				
P Content and Digestibility	**Reference**	**Raw**	**Enzyme-Treated**	**Extruded**	**p Value[b]**
Total P (g kg^{-1}) feed[c]	37.0	23.8	21.8	25.00	–
ADC[d] (%) of P	64.8 ± 1.1 [a]	63.6 ± 1.3 [a]	63.0 ± 0.8 [ab]	57.4 ± 1.2 [b]	0.0162
Digestible P (g kg^{-1})	24.0 ± 0.4 [a]	15.2 ± 0.3 [b]	13.8 ± 0.2 [c]	14.6 ± 0.3 [bc]	0.0001

[a]Mean values not sharing a common bracketed letter in the same row differ significantly as determined by Tukey's HSD test, $p < 0.05$; both letters appearing together means no difference.

[b]These p values are for ANOVA comparing the mean values for each diet.

[c]The diets are part of the experimental design and randomly assigned to 16 tanks, with each diet fed to 4 tank replicates. The statistical design did not consider the biochemical composition (total P) of the diet.

[d]Apparent digestibility coefficient.

Table 4. Quantity and digestibility of phosphorus for each microalgal co-product experimental ingredient (*Nannochloropsis oculata* raw, enzyme-treated, and extruded; mean ± standard error, n = 4)

	N. oculata Co-Product Ingredient[a]			
P Content and Digestibility	**Raw**	**Enzyme-Treated**	**Extruded**	**p Value[b]**
Total P (g kg^{-1}) ingredient[c]	17.0	19.0	17.0	–
ADC (%) of P	57.5 ± 4.8 [a]	54.7 ± 8.7 [a]	30.9 ± 8.8 [a]	0.0705
Digestible P (g kg^{-1})	13.7 ± 1.2 [a]	11.9 ± 1.9 [a]	7.7 ± 2.2 [a]	0.1077

[a]Mean values not sharing a common bracketed letter in the same row differ significantly as determined by Tukey's HSD test, $P < 0.05$; both letters appearing together means no difference.

[b]These p values are for ANOVA comparing the mean values for each diet.

[c]The diets are part of the experimental design and randomly assigned to 16 tanks, with each diet fed to 4 tank replicates. The statistical design did not consider the biochemical composition (total P) of the diet ingredients.

Table 5. Phosphorus budget fractions (mean ± standard error, n = 4[a]) in the reference diet and test diets for rainbow trout

	Diets[b]				
P Budget Fraction	**Reference**	**Raw**	**Enzyme-Treated**	**Extruded**	**p Value[c]**
Intake P (g kg^{-1})	92.6 ± 7.74 [a]	56.7 ± 5.20 [ab]	42.4 ± 0.75 [b]	73.9 ± 13.4 [ab]	0.0127
P retention (%)	26.0 ± 4.94 [b]	44.4 ± 6.55 [ab]	70.9 ± 4.94 [a]	37.0 ± 13.11 [ab]	0.0429
Total P load (g kg^{-1})	69.6 ± 9.98[a]	32.1 ± 5.25 [ab]	12.3 ± 4.39 [b]	50.6 ± 15.5 [ab]	0.0184
Solid P (g kg^{-1})	32.5 ± 2.87 [a]	20.6 ± 2.10 [a]	15.8 ± 0.15 [a]	31.0 ± 0.010 [a]	0.0332
Dissolved P (g kg^{-1})	37.0 ± 7.51[a]	11.5 ± 4.28 [ab]	−3.48 ± 4.33 [b]	19.6 ± 9.57 [ab]	0.0174

[a]The enzyme-treated diet was analyzed with 3 replicates (n = 3).

[b]Mean values not sharing a common bracketed letter in the same row differ significantly as determined by Tukey's HSD test, $p < 0.05$; both letters appearing together means no difference.

[c]These p values are for ANOVA comparing the mean values for each diet.

$p = 0.04$), total P load ($F_{3,11} = 5.14$, $p = 0.02$), and dissolved P ($F_{3,11} = 5.23$, $p = 0.02$).

The intake P was greatest in trout fed the reference diet (92.6 g P kg^{-1}) and lowest in those fed the enzyme diet (42.4 g P kg^{-1}). The percentage of P retained was greatest in trout fed the enzyme diet (70.9%) and lowest in those fed the reference diet (26.0%). The total P load was greatest from trout fed the reference diet (69.6 g P kg^{-1}) and lowest from trout fed the enzyme diet (12.3 g P kg^{-1}).

Table 6. Nitrogen budget fractions (mean ± standard error, n = 4[a]) in the reference diet and test diets for rainbow trout

	Diets[b]				
N Budget Fraction	Reference	Raw	Enzyme-Treated	Extruded	p Value[c]
Intake N (g kg^{-1})	161 ± 13.45	169 ± 13.5	135 ± 2.39	216 ± 39.16	0.178
N retention (%)	46.7 ± 5.45	55.17 ± 5.32	74.9 ± 5.94	47.0 ± 10.92	.096
Total N load (g kg^{-1})	88.0 ± 15.9	77.7 ± 14.1	33.5 ± 7.35	127 ± 43.2	0.174
Solid N (g kg^{-1})	8.17 ±1.05	14.9 ± 1.17	10.9 ± 0.43	19.3 ± 4.83	0.63
Dissolved N (g kg^{-1})	79.8 ± 15.01	62.8± 13.0	22.6 ± 7.40	107.7 ± 38.8	0.164

[a]The enzyme-treated diet was analyzed with 3 replicates (n = 3).

[b]Mean values not sharing a common bracketed letter in the same row differ significantly as determined by Tukey's HSD test, $p < 0.05$; both letters appearing together means no difference.

[c]These p values are for ANOVA comparing the mean values for each diet.

The dissolved P was greatest in trout fed the reference diet (37.02 g P kg^{-1}) and lowest in those fed the enzyme diet, where we measured a negative value (−3.48 g P kg^{-1}). There was no difference in solid excreted P (g kg^{-1}) for fish fed on different diets. However, the enzyme-treated diet had the lowest average value of all test diets (15.81 g P kg^{-1}).

N budget fractions

We report N budget fractions for trout fed different diets in **Table 6**. We did not find any significant differences ($p > 0.05$) between treatments in intake N, N retention, total N load, solid excreted N, or dissolved N.

The intake of N was highest in the trout that were fed the extruded diet (216 g N kg^{-1}) and lowest in the trout that were fed enzyme-treated diet (135 g N kg^{-1}). The percentage of N retained was greatest in the trout that were fed the enzyme-treated diet (74.9%) and lowest in the trout that were fed the reference diet (46.7%). The total N load was greatest in the trout that were fed the extruded diet (127 g N kg^{-1}) and lowest in the trout that were fed the enzyme-treated diet (33.5 g N kg^{-1}).

The dissolved N was greatest in the extruded diet (107.7 g N kg^{-1}) and lowest in the enzyme treated diet (22.6 g N kg^{-1}). The solid excreted N was highest in the extruded diet (19.3 g N kg^{-1}) and lowest in the reference diet (8.17 g N kg^{-1}).

Discussion

Here we highlight key results of this study, particularly with regard to the enzyme-treated diet, and their relevance to future sustainability research and decisions. There were no significant differences in trout growth and weight gain among all diets tested. Additionally, SGR and FCR values did not vary between the reference and enzyme-treated diets. The similar FCR values between these two diets indicate that the protein and energy content of the diets were balanced (Cho and Kaushik, 1990). Also, rainbow trout fed with the test diets showed no significant differences in N retention and excretions compared to the reference feed. This result indicates that all the test diets, including the enzyme-treated diet, had adequate amounts of protein to support equal growth and, consequently, equal metabolic N waste output. FCR values in this study are slightly greater than the range reported from a previous study that compared diets fully replaced with microalgae to the conventional reference diet of fish meal and fish oil (Sarker et al., 2020). Despite this difference, our results remain at an FCR value close to 1 and thus encourage the use of diets supplemented with processed *Nannochloropsi oculata* biomass. There are limited studies available on the effect of diets incorporating *Nannochloropsis* spp. on growth, digestibility, and feed utilization of salmonids including rainbow trout (Sørensen et al., 2017; Gong et al., 2018; Sarker et al., 2020). Most of these studies revealed that *Nannochloropsis* co-product biomass is a good source of protein for salmonids and could be a potential protein ingredient for aqua-feed formulations.

Although trout fed the reference diet had the highest ADC of P, the budget fractions revealed that they had the lowest retained P, the highest P load into the environment, and the highest levels of dissolved and solid P. These results suggest that the trout were able to digest the higher amount of P intake from the reference diet but then excreted excess amounts of solid and dissolved forms of P via feces, kidney, and gills. Studies have shown that traditional trout diets, which include fish meal, fish oil, and terrestrial crops, result in excess P excretion due to indigestible forms of P found in these ingredients (Sarker et al., 2011; Gamble et al., 2021).

The trout fed the raw co-product diet had a significantly higher ADC of P compared to the extruded diet, indicating that extrusion processing may not be sufficient to increase the P digestibility of microalgal co-product ingredients enough to make a useful difference. The indigestible complex NSP, which are mostly associated with the leftover rigid cell walls, remained in the co-product, yet NSP should be kept lower specifically in trout feeds because they might inhibit the digestibility of nutrients including P (Scholz et al., 2014; Gong et al., 2018; Sarker et al., 2018). This effect suggests that further improvements are needed to make the

P more digestible and accessible to the trout. It may also suggest that other methods such as enzymatic treatment could be used to increase nutrient digestibility and accessibility of nutrients from *Nannochloropsi oculata* co-product.

Fish fed the enzyme-treated co-product diet had a P digestibility of 63.0%, which was similar to the reference and extruded diet, but slightly lower than the values of 70%–74% reported by Dalsgaard et al. (2012). Previous studies have shown that adding exogenous NSP enzymes to salmonid diets containing terrestrial crop meal can aid in reducing antinutritional factors, including phytate-bound P, and improve FCR and P digestibility (Dalsgaard et al., 2012; Dalsgaard et al., 2016; Maas et al., 2019). How NSP enzymes might improve P digestibility in salmonid diets containing microalgal co-product, however, is uncertain.

The enzyme-treated diet resulted in the highest P retention, the lowest total P load to the environment with a negative value, and the lowest levels of solid and dissolved P excretion. Although our growth results suggest the enzyme-treated diet contained sufficient nutrients to fulfill minimum requirements for rainbow trout, negative values for dissolved P loading suggest that this diet was deficient in bioavailable P and that the trout may have absorbed more P through their gills to meet their requirements (Sugiura et al., 2003; Sarker et al., 2014; Gamble et al., 2021). The lower P excretion and higher retention in rainbow trout fed with enzyme-treated diets may be due to their lower P intake compared to other diets. The findings of this study that indicate a negative value in terms of dissolved P loading may be helpful for fish nutritionists in improving future formulations for nutritional feeding experiments to enhance feed P intake. The goal is to incorporate slightly elevated levels of enzyme-treated co-product as a substitute for fishmeal in the diet while ensuring that rainbow trout receive sufficient amounts of bioavailable P. The successful use of enzyme-treated microalgal co-product meal can add to the variety of ingredients available to feed suppliers and trout producers interested in sustainable diets.

Determining dissolved P excretion in microalgal co-product diets is necessary to formulate trout feeds with optimal P levels. The high ADC of P and negative dissolved P levels from the enzyme-treated diet suggest that this diet would need more fine-tuning in its formulation to ensure a sufficient level of digestible/bioavailable P. Elevated dissolved P levels in tanks where fish were fed the fishmeal-based reference feed likely resulted from P that was not required by the trout and therefore excreted into the water. Pursuing such fine tuning is important for two reasons. First, excreted dissolved P from aquaculture contributes to eutrophication as the dissolved P is available to other aquatic organisms, increasing their productivity. Second, removal of dissolved P wastes from aquaculture systems is a more intractable problem than solid P-waste removal. Techniques designed to remove dissolved P in fish culture systems are not effective (Ruzhitskaya and Gogina, 2017). Dissolved P loads depend on feed P levels, and how efficiently fish digest and retain the feed (Koko et al., 2010; Sarker et al., 2011; Sarker et al., 2014).

Our team is currently investigating a nutritional strategy to decrease eutrophication potential in aquaculture feeds. The dissolved P loading results of the fishmeal-based reference feeds indicate that inclusion of inorganic phosphate could be significantly reduced in fishmeal-based diets without affecting the growth of larger trout (Sarker et al., 2014). To achieve this goal, we are focusing on developing aquaculture feeds that incorporate under-utilized microalgal co-products to minimize eutrophication emissions from trout aquaculture by adjusting the levels of inorganic phosphate inclusion in trout feed.

The majority of industrial algal protein players are still early in commercialization. Although microalgae have high potential as a protein source, the technical, biological, and economic difficulties regarding the continuous production of high-quality microalgal biomass and its downstream processing diminish this potential at scale (Nagappan et al., 2021). Our industry collaborator, Qualitas Health Inc., a US leader in marketing EPA-rich oil extracted from *Nannochloropsi oculata* as a human supplement, seeks uses for large amounts of co-product from its large-scale production of *N. oculata* and will provide us co-product for this research. However, for the further development and commercial adoption of microalgal co-product ingredients for farmed salmonid feeds there is a need for additional technological advancements in the areas of industrial algaculture scale-up, standardization of cultivation strategies and down-stream processing methods to concentrate nutrient levels and increase their nutrient bioavailability (Hoffman et al., 2017). These advancements should enable the industry to provide nutrient-dense, highly digestible microalgae-based ingredients at cost-competitive prices.

Conclusions

Juvenile rainbow trout fed a diet that included enzymatically processed microalgal co-product displayed the highest (but not statistically different) growth of other feeds tested, followed by comparable values for P digestibility and solid P excretion to the reference diet. Trout fed the enzyme-treated diet had the lowest total P and dissolved P loading (with a negative value for P loading) while the reference diet had the highest. The high levels of P loading produced by the reference diet may put aquaculture operations at a disadvantage.

Results of this study are an important step toward developing an economically and environmentally sustainable nutritional strategy to decrease the potential for eutrophication caused by diets used in current fed aquaculture. For example, the dissolved P loading results of the fishmeal-based reference feeds indicate that inclusion of inorganic phosphate should be significantly reduced in fishmeal-based diets, and could be without affecting the growth of larger trout (Sarker et al., 2014). Our team is currently investigating a nutritional strategy to decrease eutrophication potential in aquaculture feeds. To achieve this goal, we are focusing on developing aquaculture feeds that incorporate under-utilized microalgal co-products to minimize eutrophication emissions from trout aquaculture by adjusting the levels of inorganic phosphate inclusion in trout feed.

This study demonstrated reduction of solid and dissolved P emissions by rainbow trout when provided with a diet that contained an enzyme-treated microalgal co-product. The results indicate that microalgal feeds treated with exogenous enzymes could be applied in aquaculture feeds to improve P digestibility and utilization and reduce the potential for pollution. However, studies on life cycle environmental impacts (global warming and resource use) and the economic viability of using exogenous enzymes to process microalgal co-product for aquaculture diets are still needed (Mckuin et al., 2023). We are now focusing on life cycle environmental analysis and techno-economic analysis of marine microalgae-based feed for aquaculture.

Data accessibility statement

All data generated for this study are included in the article and the tables.

Acknowledgments

The authors would like to thank the University of California Santa Cruz, Dean of Social Sciences and Executive Vice Chancellor for construction and set up of the Ecological Aquaculture research facility. They also thank Rebecca White at Qualitas Health, Inc. for donating under-utilized *Nannochloropsis oculata* defatted biomass for this research.

Funding

This work was made possible by funding from a California Sea Grant Award (grant no. NA18OAR4170073); Agriculture and Food Research Initiative Competitive Grant award no. 2021-67016-33394 from the USDA NIFA; and Agriculture and Food Research Initiative Competitive Grant no. 2021-69014-34501 from the USDA NIFA.

Competing interests

No competing interests. Coauthor Dr. Kapuscinski is a former Editor-in-Chief for the Elementa Sustainability Transitions domain, but was not involved at all in handling or reviewing this article.

Author contributions

Conceived and designed the experiments: PKS, ARK.

Performed the experiments: DF, SA, CG, PN, KO'S, BL, AM, DG, DGO, LW.

Analyzed the data: PKS.

Contributed reagents/materials/analysis tools: DF, SA, BS, US.

Wrote original draft: SA, PKS.

Wrote, reviewed and edited: SA, PKS, DF, BS, US, CG, PN, KO'S, BL, AM, DG, DGO, LW, ARK.

References

Association of Official Analytical Chemists. 1995. Agriculture chemicals; contaminants, drugs, in Latimer, GW, Horwitz, W eds., *Official methods of analysis of AOAC International*, 16th ed. Vol. I. Arlington, VA: AOAC International.

Barrows, FT, **Stone, DAJ**, **Hardy, RW.** 2007. The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **265**(1–4): 244–252. DOI: http://dx.doi.org/10.1016/j.aquaculture.2007.01.017.

Bélanger, A, **Sarker, PK**, **Bureau, DP**, **Chouinard, Y**, **Vandenberg, GW.** 2021. Apparent digestibility of macronutrients and fatty acids from microalgae (*Schizochytrium* sp.) fed to rainbow trout (*Oncorhynchus mykiss*): A potential candidate for fish oil substitution. *Animals* **11**(2): 456. DOI: http://dx.doi.org/10.3390/ani11020456.

Bureau, DP, **Hua, K.** 2006. Letter to the Editor of *Aquaculture*. *Aquaculture* **252**(2–4): 103–105. DOI: http://dx.doi.org/10.1016/j.aquaculture.2006.01.028.

Bureau, DP, **Hua, K.** 2010. Towards effective nutritional management of waste outputs in aquaculture, with particular reference to salmonid aquaculture operations. *Aquaculture Research* **41**(5): 777–792.

Cho, C, **Kaushik, S.** 1990. Nutritional energetics in fish: Energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Review of Nutrition and Dietetics* **61**: 132–172. DOI: http://dx.doi.org/10.1159/000417529.

Cho, CY, **Slinger, SJ**, **Bayley, HS.** 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **73**(1): 25–41. DOI: http://dx.doi.org/10.1016/0305-0491(82)90198-5.

Choct, M. 1997. Feed non-starch polysaccharides: Chemical structures and nutritional significance. *Feed Milling International* **191**(1): 13–26.

Dalsgaard, J, **Bach Knudsen, KE**, **Verlhac, V**, **Ekmann, KS**, **Pedersen, PB.** 2016. Supplementing enzymes to extruded, soybean-based diet improves breakdown of non-starch polysaccharides in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition* **22**(2): 419–426.

Dalsgaard, J, **Verlhac, V**, **Hjermitslev, NH**, **Ekmann, KS**, **Fischer, M**, **Klausen, M**, **Pedersen, PB.** 2012. Effects of exogenous enzymes on apparent nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) fed diets with high inclusion of plant-based protein. *Animal Feed Science and Technology* **171**(2–4): 181–191.

Food and Agriculture Organization. 2020. *The state of world fisheries and aquaculture 2020. Sustainability in action*. Rome, Italy: Food and Agriculture Organization of the United Nations.

Gamble, MM, **Sarker, PK**, **Kapuscinski, AR**, **Kelson, S**, **Fitzgerald, DS**, **Schelling, B**, **De Souza, AVB**, **Tsukui, T.** 2021. Toward environmentally sustainable aquafeeds: Managing phosphorus discharge from Nile tilapia (*Oreochromis niloticus*) aquaculture with microalgae-supplemented diets. *Elementa: Science of the Anthropocene* **9**(1): 00170.

Glencross, BD, **Booth, M**, **Allan, GL.** 2007. A feed is only as good as its ingredients? A review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition* **13**(1): 17–34. DOI: http://dx.doi.org/10.1111/j.1365-2095.2007.00450.x.

Gong, Y, **Guterres, HADS**, **Huntley, M**, **Sørensen, M**, **Kiron, V.** 2018. Digestibility of the defatted microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. when fed to Atlantic salmon, *Salmo salar*. *Aquaculture Nutrition* **24**(1): 56–64.

Hoffman, J, **Pate, RC**, **Drennen, T**, **Quinn, JC.** 2017. Techno-economic assessment of open microalgae production systems. *Algal Research* **23**: 51–57.

Jobling, M. 2011. National Research Council (NRC): Nutrient requirements of fish and shrimp. *Aquaculture International* **20**: 601–602. DOI: http://dx.doi.org/10.1007/s10499-011-9480-6.

Kiron, V, **Phromkunthong, W**, **Huntley, M**, **Archibald, I**, **De Scheemaker, G.** 2012. Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. *Aquaculture Nutrition* **18**(5): 521–531. DOI: http://dx.doi.org/10.1111/j.1365-2095.2011.00923.x.

Koko, GKD, **Sarker, PK**, **Proulx, É**, **Vandenberg, GW.** 2010. Effects of alternating feeding regimes with varying dietary phosphorus levels on growth, mineralization, phosphorus retention and loading of large rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources* **23**(3): 277–284. DOI: http://dx.doi.org/10.1051/alr/2010032.

Maas, RM, **Verdegem, MC**, **Schrama, JW.** 2019. Effect of non-starch polysaccharide composition and enzyme supplementation on growth performance and nutrient digestibility in Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* **25**(3): 622–632.

Mainstone, CP, **Parr, W.** 2002. Phosphorus in rivers—Ecology and management. *Science of the Total Environment* **282–283**: 25—47.

McKuin, B, **Kapuscinski, AR**, **Sarker, PK**, **Cheek, N**, **Lim, J**, **Sabarsky, M.** 2023. Comparative life cycle assessment of marine microalgae, *Nannochloropsis* sp. and fishmeal for sustainable protein ingredients in aquaculture feeds. *Elementa: Science of the Anthropocene* **11**(1): 00083. DOI: http://dx.doi.org/10.1525/elementa.2022.00083.

Nagappan, S, **Das, P**, **AbdulQuadir, M**, **Thaher, M**, **Khan, S**, **Mahata, C**, **Al-Jabri, H**, **Vatland, AK**, **Kumar, G**. 2021. Potential of microalgae as a sustainable feed ingredient for aquaculture. *Journal of Biotechnology* **341**: 1—20. DOI: https://doi.org/10.1016/j.jbiotec.2021.09.003.

National Research Council. 1993. *Nutrient requirements of fish*. Washington, DC: The National Academies Press.

Ruzhitskaya, O, **Gogina, E**. 2017. Methods for removing of phosphates from wastewater, in SPbWOSCE-2016, *MATEC Web of Conferences* 106: 07006.

Sarker, PK, **Bureau, DP**, **Hua, K**, **Drew, MD**, **Forster, I**, **Were, K**, **Hicks, B**, **Vandenberg, GW.** 2013. Sustainability issues related to feeding salmonids: A Canadian perspective. *Reviews Aquaculture* **5**(4): 199—219.

Sarker, PK, **Fournier, J**, **Boucher, E**, **Proulx, E**, **de la Noüe, J**, **Vandenberg, GW.** 2011. Effects of low phosphorus ingredient combinations on weight gain, apparent digestibility coefficients, non-fecal phosphorus excretion, phosphorus retention and loading of large rainbow trout (*Oncorhynchus mykiss*). *Animal Feed Science and Technology* **168**(3–4): 241–249.

Sarker, PK, **Gamble, MM**, **Kelson, S**, **Kapuscinski, AR.** 2016. Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium* sp. and of protein and essential amino acids from freshwater *Spirulina* sp. feed ingredients. *Aquaculture Nutrition* **22**(1): 109–119. DOI: http://dx.doi.org/10.1111/anu.12230.

Sarker, PK, **Kapuscinski, AR**, **Bae, AY**, **Donaldson, E**, **Sitek, AJ**, **Fitzgerald, DS**, **Edelson, OF.** 2018. Towards sustainable aquafeeds: Evaluating substitution of fishmeal with lipid-extracted microalgal co-product (*Nannochloropsis oculata*) in diets of juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS One* **13**(7): e0201315. DOI: http://dx.doi.org/10.1371/journal.pone.0201315.

Sarker, PK, **Kapuscinski, AR**, **Vandenberg, GW**, **Proulx, E**, **Sitek, AJ.** 2020. Towards sustainable and ocean-friendly aquafeeds: Evaluating a fish-free feed for rainbow trout (*Oncorhynchus mykiss*) using three marine microalgae species. *Elementa: Science of the Anthropocene* **8**: 5.

Sarker, PK, **Pilote, A**, **Auffret, M**, **Proulx, É**, **Villemur, R**, **Deschamps, MH**, **Vandenberg, G.** 2014. Reducing geosmin off-flavor compounds and waste outputs through dietary phosphorus management in rainbow trout aquaculture. *Aquaculture Environment Interactions* **6**(1): 105–117.

Scholz, MJ, **Weiss, TL**, **Jinkerson, RE**, **Jing, J**, **Roth, R**, **Goodenough, U**, **Posewitz, MC**, **Gerken, HG.** 2014. Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. *Eukaryotic Cell* **13**(11): 1450–1464.

Sørensen, M, **Gong, Y**, **Bjarnason, F**, **Vasanth, GK**, **Dahle, D**, **Huntley, M**, **Kiron, V**. 2017. *Nannochloropsis oceania*-derived defatted meal as an alternative to fishmeal in Atlantic salmon feeds. *PLoS One* **12**(7): e0179907. DOI: http://dx.doi.org/10.1371/journal.pone.0179907.

Sugiura, SH. 2018. Phosphorus, aquaculture, and the environment. *Reviews in Fisheries Science & Aquaculture* **26**(4): 515–521.

Sugiura, SH, **McDaniel, NK**, **Ferraris, RP.** 2003. In vivo fractional Pi absorption and NaPi-II mRNA expression in rainbow trout are upregulated by dietary P restriction. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **285**(4): R770–R781.

Sugiura, SH, **Raboy, V**, **Young, KA**, **Dong, FM**, **Hardy, RW.** 1999. Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **170**(3–4): 285–296.

Suresh, K, **Tang, T**, **Vliet, MTH**, **Bierkens, MFP**, **Strokal, M**, **Sorger-Domenigg, F**, **Wada, F**. 2023. Recent advancement in water quality indicators for

eutrophication in global freshwater lakes. *Environmental Research Letters* **18**(6): 063004. DOI: http://dx.doi.org/10.1088/1748-9326/acd071.

Tibbetts, SM, **Yasumaru, F**, **Lemos, D.** 2017. In *vitro* prediction of digestible protein content of marine microalgae (*Nannochloropsis granulata*) meals for Pacific white shrimp (*Litopenaeus vannamei*) and rainbow trout (*Oncorhynchus mykiss*). *Algal Research* **21**: 76–80. DOI: http://dx.doi.org/10.1016/j.algal.2016.11.010.

How to cite this article: Andrade, S, Sarker, PK, Kapuscinski, AR, Fitzgerald, D, Greenwood, C, Nocera, P, O'Shelski, K, Lee, B, Mkulama, A, Gwynne, D, Orcajo, DG, Schoffstall, B, Sarker, U, Warkaw, L. 2024. Enzyme-treated microalgal co-product diets for rainbow trout aquaculture: Supporting fish growth, phosphorus digestibility, and reducing phosphorus waste emission. *Elementa: Science of the Anthropocene* 12(1). DOI: https://doi.org/10.1525/elementa.2023.00119

Domain Editor-in-Chief: Jody W. Deming, University of Washington, Seattle, WA, USA

Associate Editor: Laurenz Thomsen, Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden

Knowledge Domain: Ocean Science

Published: May 09, 2024 **Accepted:** March 17, 2024 **Submitted:** September 25, 2023

ELEMENTA Science of the Anthropocene

Elem Sci Anth is a peer-reviewed open access journal published by University of California Press.

OPEN ACCESS