1-15-2024

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Recommended Citation

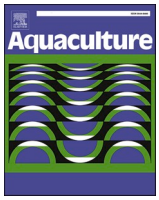
Katherine A. Neylan, Ronald B. Johnson, Frederic T. Barrows, David P. Marancik, Scott L. Hamilton, and Luke D. Gardner. "Evaluating a microalga (Schizochytrium sp.) as an alternative to fish oil in fish-free feeds for sablefish (Anoplopoma fimbria)" *Aquaculture* (2024). https://doi.org/10.1016/j.aquaculture.2023.740000

Aquaculture 578 (2024) 740000

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Evaluating a microalga (*Schizochytrium* sp.) as an alternative to fish oil in fish-free feeds for sablefish (*Anoplopoma fimbria*)

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ARTICLE INFO

Keywords:
Aquaculture
Fish-free feeds
Alternative feeds
Microalgae
Fish oil replacement

ABSTRACT

Alternative feeds are critical for the sustainable expansion of the marine finfish aquaculture industry. The industry uses wild-caught forage fish as a primary ingredient in farmed fish feeds. Alternative ingredients are needed to safeguard fisheries' sustainability and future aquaculture development. While there have been successes in alternative feeds, it is necessary to improve the existing options and identify alternative ingredients with higher concentrations of omega-3 polyunsaturated fatty acids (PUFAs). This study was designed to test a microalga, *Schizochytrium* sp., as a feed ingredient for sablefish (*Anoplopoma fimbria*) using six test diets. There were two fish-ingredient control diets: +FM+FO, which contained both fishmeal and fish oil, and −FM+FO, which contained fish oil, but no fishmeal. The remaining four diets contained alternative lipids and were completely fish-free. FF Flax contained flax oil as the only lipid source replacing fish oil. FF LowSc, FF ModSc, and FF HighSc contained a low, moderate, and high level of *Schizochytrium* sp. to replace fish oil, with flax oil content decreasing as the microalga increased. After a 20-week trial, sablefish growth differed across the feed treatments, with fish fed the high microalga-inclusion diet (FF HighSc) performing similarly to fish fed the fish-ingredient controls. Fulton's K condition factor, dry feed intake (DFI), and lipid productive value (LPV) were also influenced by treatment. For the four fish-free diets, specific growth rate increased with increasing inclusion of *Schizochytrium* sp. in the feed. Fillet fatty acid profiles were similarly influenced by diet treatment, generally reflecting the fatty acid profiles of the feed. Total fillet PUFAs were higher in sablefish from the fish-free treatments than the control treatments, with DHA increasing with increasing inclusion of dietary *Schizochytrium*. In contrast, EPA was higher in fillets from both fish-ingredient control treatments compared to fillets from the fish-free treatments, yet EPA remained higher than expected in sablefish fed the fish-free diets. Histologic evaluation of sablefish distal intestine and liver demonstrated that the microalga-inclusion diets were well tolerated and did not cause histomorphological changes in the tissues. These results suggest *Schizochytrium* sp. can increase PUFA concentrations in fish fillets without compromising fish health and growth, making it a viable ingredient for alternative sablefish feeds.

1. Introduction

Cultured marine fish are largely dependent on wild-caught forage fish as the primary nutrition source, which are incorporated in feeds in the form of fishmeal (FM) and fish oil (FO). These major ingredients are generally considered to contain the proper nutrient requirements for fish growth, are readily digestible, and have high levels of long-chain polyunsaturated fatty acids (PUFAs) (FAO, 2016; Rust et al., 2011). In 2018, approximately 18 million metric tons of global fisheries landings were used to produce fishmeal (FM) and fish oil (FO) for aquaculture (FAO, 2020). While FM and FO are high quality feed ingredients, the supply is variable due to natural climate variability and heavy fishing pressure, resulting in decreasing landings since 1994; together this has led to an increase in the price for these ingredients (FAO, 2018).

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https://doi.org/10.1016/j.aquaculture.2023.740000
Received 30 March 2023; Received in revised form 17 July 2023; Accepted 15 August 2023
Available online 19 August 2023

Alternative feeds are necessary if the aquaculture industry is to meet projected seafood demands. Terrestrial sources of protein (i.e., soybean products, corn protein concentrate, poultry meal, etc.) have been studied as alternatives to FM for cultured carnivorous fish species for over 40 years, and these ingredients have been increasingly incorporated into commercial feeds (Ayadi et al., 2012; Tacon and Metian, 2015). However, FO has been more difficult to replace in marine fish feeds due to specific polyunsaturated fatty acid (PUFA) nutritional requirements. While some terrestrial sources of oil contain short chain omega-3 PUFAs, they do not contain all of the PUFAs required by fish, specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These omega-3 fatty acids are abundant in wild-caught marine fish, can benefit fish growth and human health, and are expected to be present in cultured fish by human consumers.

DHA and EPA are considered essential omega-3 PUFAs since marine fish are incapable of *de novo* production of these fatty acids, therefore DHA and EPA must be included in the feeds for fish health and growth (National Research Council, 1993; National Research Council, 2011). DHA and EPA are highly unsaturated fatty acids (HUFAs), which is a subset of PUFAs that have 20 or more carbon atoms in their structure. These omega-3 HUFAs have well-documented human health benefits (Ruxton et al., 2005; Shahidi and Miraliakbari, 2004; Simopoulos, 2002). In contrast, excessive dietary omega-6 fatty acids (which are comparatively higher in terrestrial ingredients), without sufficient omega-3 fatty acids can result in inflammation in the human body and other precursors for disease (Simopoulos, 2002). Since fish are a primary source of omega-3 fatty acids for human consumers, it is important to assess the omega-3 (ω-3) to omega-6 (ω-6) ratio of cultured fish when developing alternative feeds.

Many of the alternative lipid feed studies to date have investigated partial replacement of FO, while few have attempted complete replacement of FO in marine finfish diets (Turchini et al., 2009, 2019). Flax oil is one alternative lipid that has been studied as a FO replacement in sablefish feeds (Friesen et al., 2013a, 2013b). Friesen et al. (2013a) found that flax oil could replace up to 75% of FO in juvenile sablefish feeds without compromising growth and other performance metrics; however, DHA and EPA levels in fillets declined as flax oil inclusion increased. Complete replacement of FO with flax oil was not tested. Other terrestrially sourced lipid alternatives have resulted in similar outcomes with comparably low final HUFA concentrations in fillets, with the exception of a genetically modified canola oil (Betancor et al., 2017; Ruyter et al., 2019).

To produce cultured fish with fatty acid profiles more similar to their wild counterparts, alternative feeds need to be developed that contain sufficient levels of omega-3 HUFAs. Certain microalgae species have recently been considered for inclusion in alternative aquaculture feeds due to their high HUFA content. Recent studies have reported favorable growth in freshwater fish and rainbow trout when FO was partially and fully replaced with microalgal lipid sources (Sarker et al., 2016, 2018; Serrano et al., 2021). A preliminary feeding study with juvenile sablefish reported similar growth when FO was replaced with flax or corn oils supplemented with specialty microalgae containing DHA (Goetz et al., 2021). Further research is needed to evaluate promising microalgae species as feed ingredients for commercially important fish species in the aquaculture industry. *Schizochytrium* sp. is a commercially grown heterotrophic microalga that is a promising candidate for FO replacement in aquaculture feeds (Carter et al., 2003; Miller et al., 2007; Sarker et al., 2016). This microalga is rich in DHA and low in EPA, a common characteristic of the currently available commercial microalgae products.

Sablefish (*Anoplopoma fimbria*) is a relatively new species for the aquaculture industry that is increasing in popularity. This species is a potential candidate for aquaculture in temperate U.S. waters, as it is a native cold-water species, can withstand high stocking densities, and has one of the highest documented growth rates of teleost fishes during its juvenile stage (Sogard and Olla, 2001; Sogard and Spencer, 2004). Sablefish flesh is high in oil content, making them a valued species with large markets in Japan, Russia, and the United States (FAO, 2016; Warpinski et al., 2016). In an aquaculture setting, sablefish can grow from larvae to a harvestable size (2.5 kg) in 36 months, or as fast as 24 months if raising all females (Luckenbach et al., 2017).

There have been relatively few studies on sablefish nutrition (Forster et al., 2017; Friesen et al., 2013a, 2013b; Goetz et al., 2021; Johnson et al., 2015; Reid et al., 2017; Rhodes et al., 2016) considering the increasing interest in this species for aquaculture. As such, there is a need to expand the understanding of sablefish nutritional requirements and identify viable alternative feed ingredients for this species to support its sustainable growth within the aquaculture industry. While diets have not yet been optimized for sablefish, they grow well on commercial salmon diets, which range from 42% to 45% protein and 15% to 33% lipid, and a number of feed studies have been designed following these protein and lipid guidelines (Goetz et al., 2021). Of these nutrition studies, most have been conducted with small juvenile fish (between 5 g and 100 g starting weight). Juvenile fish grow relatively fast, permitting a rapid assessment of nutritional demands, but nutritional requirements often differ between grow-out and juvenile stages (National Research Council, 2011).

In this study, a series of diets were designed to investigate the effects of *Schizochytrium* sp. dried, whole-cell meal on sub-adult sablefish performance. Specifically, we sought to (1) examine the effects of low, moderate, and high inclusion of *Schizochytrium* sp. on sablefish growth, condition, nutrient utilization, and digestive tissue health and (2) determine if sablefish can incorporate omega-3 fatty acids from the *Schizochytrium* sp. dried meal into their fillets. This study provides a needed analysis of a novel ingredient for the aquaculture industry to successfully transition to fish-free alternative feeds for sablefish culture.

2. Methods

2.1. Feed formulation

Six experimental diets were formulated to be iso-nitrogenous (46% protein) and isolipidic (15% lipid) using Creative Formulation Concepts software (Table 1). The reference diet (+FM+FO) was formulated based on the composition of a commercial aquaculture feed with standard levels of FM and FO. The FO control (−FM+FO) contained the standard amount of FO and a combination of poultry meal, soy protein concentrate, and corn protein concentrate to replace FM. The remaining four diets were completely fish-free (FF) and contained the same FM replacement ingredients as −FM+FO, but differed in the lipid composition. FF Flax contained flax oil as the only lipid source (0% *Schizochytrium* sp.). The microalga-inclusion diets (FF LowSc, FF ModSc, and FF HighSc) contained low (4%), moderate (8%), and high (12%) levels of *Schizochytrium* sp. meal (Corbion, San Francisco, CA, USA) at the expense of flax oil. The pellets were slow-sinking and were extruded with a small twin screw extruder using industry standard techniques by Zeigler Bros., Inc. in PA, USA.

The *Schizochytrium* sp. meal was analyzed by NP Analytical Laboratories (St. Louis, Missouri, USA) for proximate analysis (Table 2) and complete fatty acid profiles (Table 3). Crude protein, moisture, fiber, and ash were measured using standard methods (990.03, 930.15, 962.09, and 942.05, respectively) by the Association of Official Analytical Chemists (AOAC International, 2019). Total fat (total fatty acids) and the concentration of individual fatty acids (% of total fatty acids) were also determined following AOAC methodology (996.06).

The test diets were analyzed by University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories for proximate and fatty acid analysis to verify the composition of each diet (Tables 2 & 3). Crude protein (990.03), crude fat (920.39), and crude ash (942.05) of the test diets were determined following AOAC methods (AOAC International, 2019). Moisture and crude fiber of the test diets were analyzed following American Oil Chemists' Society (AOCS) procedures (Am 5-04 and BA 6a-05, respectively). Fatty acid methyl esters were prepared

Table 1
Ingredient composition (g/100 g) of the test diets.

	+FM+FO	−FM+FO	FF Flax	FF LowSc	FF ModSc	FF HighSc
Fish meal	24.00	0.00	0.00	0.00	0.00	0.00
Poultry meal	19.92	36.12	36.12	36.12	36.12	36.12
Soy protein conc.	9.44	16.65	16.65	16.65	16.65	16.65
Corn protein conc.	7.10	5.22	5.22	5.35	5.52	5.72
Fish oil	9.65	9.65	0.00	0.00	0.00	0.00
Schizochytrium sp. meal[1]	0.00	0.00	0.00	4.00	8.00	12.00
Flax oil	0.00	0.00	9.65	7.65	5.15	2.87
Wheat flour	21.30	21.73	21.73	19.60	17.93	16.01
Dicalcium phosphate	1.86	3.40	3.40	3.40	3.40	3.10
Vitamin premix[2]	1.50	1.50	1.50	1.50	1.50	1.50
Choline CL	0.60	0.60	0.60	0.60	0.60	0.60
Lysine HCL	1.85	2.17	2.17	2.17	2.17	2.17
Methionine	0.51	0.68	0.68	0.68	0.68	0.68
Threonine	0.37	0.38	0.38	0.38	0.38	0.38
Taurine	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin C	0.20	0.20	0.20	0.20	0.20	0.20
Trace Minerals[3]	0.20	0.20	0.20	0.20	0.20	0.20

[1] AlgaPrime DHA, Corbion, San Francisco, CA, USA.
[2] ARS 702 vitamin premix.
[3] ARS 1440 trace minerals premix.

Table 2
Proximate composition of the *Schizochytrium* sp. meal and the test diets.

	Schiz.	+FM+FO	−FM+FO	FF Flax	FF LowSc	FF ModSc	FF HighSc
Moisture, %	1.3	5.1	5.5	5.9	3.9	5.1	5.1
Crude Protein, %	9.3	48.4	47.0	47.4	47.9	48.3	48.3
Total Fat, %	56.9	NA	NA	NA	NA	NA	NA
Crude fat, %	NA	15.5	14.8	14.5	13.8	14.4	14.9
Fiber, %	1.8	0.9	1.4	1.5	1.3	1.6	1.6
Ash, %	6.1	11.0	9.9	9.9	10.4	10.3	10.5

Data are presented on a dry-weight basis.

(965.49) and analyzed by capillary gas-liquid chromatography (996.06) to obtain complete fatty acid profiles of each test diet (AOAC International, 2019).

2.2. Sablefish husbandry and feeding

Cultured juvenile sablefish (65 g mean weight) were obtained from the Manchester Research Station of NOAA's Northwest Fisheries Science Center in Manchester, WA. After transport to the MLML Center for Aquaculture, juvenile sablefish were placed in an outdoor replicated tank array consisting of 18tanks (1,000 L each) and provided with flow-through seawater (11 L/min, 12 ± 2 °C), pulled from the head of the Monterey Submarine Canyon at 15 m depth, passed through sand filters, and aerated with air stones. Tanks were covered with individual shade structures (85% knitted shade cloth) to provide a suitable light environment and to prevent escape. All procedures involving sablefish in this study were reviewed and approved by the Institutional Animal Care and Use Committee (Protocol # 1068) of San José State University.

Fish were raised at the facility until they reached 334 g mean weight, and then sorted by size to exclude individuals that were too large and too small. During this time, fish were fed a 4 mm extruded pellet feed (BioBrood 4 mm, BioOregon) by hand to apparent satiation every other day. The sablefish were then fed a conditioning diet (FF Flax diet from Table 1) for ten days prior to experimentation to exclude individuals from the study that would not readily accept the test diet. During the experiment, fish were fed every other day to apparent satiation and feed intake was recorded for each tank. Water temperature was measured and recorded every day. Fish were sedated with MS-222 (40 mg/L seawater bath) on day 0 of the experiment to collect initial length and weight measurements for each fish. Tank weights (biomass of all fish in a tank) were measured for each tank every two weeks. At this time, the tanks were thoroughly cleaned.

2.3. Feed trial protocols and sampling

To test the effects of *Schizochytrium* sp. inclusion on growth and body composition, sub-adult sablefish (334 ± 41 g mean starting weight) were distributed randomly in equal numbers (15 ± 1 fish per tank) into 18 tanks. These tanks were randomly assigned to one of six treatments (three tanks per treatment). After acclimation, fish were fed their assigned treatment diets for 20 weeks (140 days) from January 2021 to May 2021 as described above and bulk tank weights were measured every two weeks (*see next section*).

On the first day of the experiment, an initial sample of 18 sablefish (one from each tank) were sacrificed with a lethal dose of MS-222 (500 mg/L seawater) and frozen at −80 °C for future analyses (*see chemical analyses section*). On day 140, all sablefish (783 g mean weight) were sacrificed, measured, and dissected. Skinned fillets were collected from five fish per tank and frozen at −80 °C to ensure preservation of the fatty acids for analyses.

Intestine and liver tissues were collected from a subsample of 12 sablefish from treatments +FM+FO, FF Flax, and FF HighSc (four fish from each replicate tank) for histological assessment to determine if diet treatment effected sablefish digestive tissue health. A representative sample (approximately 1 cm) of the distal intestine was sectioned from each fish 3 cm from the terminus at the rectum. A representative sample of liver approximately 0.5 cm in length and width was sectioned from each fish at approximately the same location. Tissues were fixed in 10% neutral buffered formalin (Fournie et al., 2000), processed routinely, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E) (Histowiz, Brooklyn, NY, USA). Slides were scanned digitally at 40× magnification using Leica AT2 scanner and three sections per tissue were examined blindly by a single pathologist using provider-based viewer software (Histowiz, Brooklyn, NY, USA).

Based on preliminary observations, each section of intestine was

Table 3
Fatty acid profile of the *Schizochytrium* sp. and the test diets – expressed as % of total fatty acids.

Fatty acid name		*Schiz.*	+FM+FO	−FM+FO	FF Flax	FF LowSc	FF ModSc	FF HighSc
Saturated fatty acids (SFAs)								
C14:0	Myristic acid	0.72	6.74	5.71	0.63	0.50	0.76	1.14
C15:0	Pentadecanoic acid	ND	0.66	0.54	0.09	0.07	0.10	0.12
C16:0	Palmitic acid	31.75	21.41	20.77	13.37	17.02	21.54	24.68
C17:0	Margaric acid	ND	0.61	0.49	0.13	0.13	0.13	0.15
C18:0	Stearic acid	1.52	4.59	4.87	5.40	5.63	4.81	4.54
C20:0	Arachidic acid	0.17	0.23	0.23	0.22	0.15	0.19	0.18
C22:0	Behenic acid	ND	0.17	0.24	0.19	0.18	0.17	0.16
Monounsaturated fatty acids (MUFAs)								
C16:1ω-7	Palmitoleic acid	0.18	10.50	9.55	2.39	2.12	2.39	2.78
C18:1ω-9	Oleic acid	0.20	13.29	16.78	23.94	20.87	18.95	15.46
C18:1 t-9	Elaidic acid	ND	0.19	0.20	0.11	0.10	0.11	0.11
C18:1ω-7	Vaccenic acid	ND	2.98	2.75	1.28	1.08	1.14	1.15
C20:1ω-9	Gondoic acid	ND	0.85	0.87	0.33	0.28	0.27	0.26
C24:1ω-9	Nervonic acid	ND	0.24	0.20	0.06	0.04	0.04	0.04
Polyunsaturated fatty acids (PUFAs)								
C18:2ω-6	Linoleic acid	ND	7.71	9.48	17.06	14.10	13.00	9.89
C18:3ω-3	a-linolenic acid	ND	1.65	2.66	30.79	27.18	16.18	10.82
C18:3ω-6	g-linolenic acid	0.14	0.20	0.18	0.06	0.03	0.10	0.13
C20:2ω-6	Eicosadienoic acid	ND	0.33	0.33	0.08	0.07	0.07	0.08
C20:3ω-6	Homo-g-linolenic acid	0.34	0.26	0.27	0.10	0.14	0.19	0.23
C20:3ω-3	Homo-a-linolenic acid	ND	0.15	0.19	0.05	0.12	0.22	0.28
C20:4ω-6	Arachidonic acid	0.15	1.15	1.14	0.55	0.57	0.61	0.68
C20:5ω-3	EPA	0.49	8.29	7.11	0.48	0.26	0.58	1.10
C22:5ω-6	Osbond acid	14.80	0.48	0.38	0.12	1.93	3.99	5.60
C22:5ω-3	Clupanodonic acid	0.12	1.51	1.37	0.13	0.09	0.16	0.26
C22:6ω-3	DHA	43.9	6.91	5.66	0.62	5.85	12.02	17.28
Summaries								
Σ SFAs		34.16	34.41	32.85	20.03	23.68	27.70	30.97
Σ MUFAs		0.38	28.05	30.35	28.11	24.49	22.90	18.80
Σ PUFAs		61.44	28.64	28.77	50.04	50.34	47.12	46.35
Σ HUFAs		61.11	19.08	16.45	2.13	9.03	17.84	25.51
Σ ω-3 HUFAs		45.83	16.86	14.33	1.28	6.32	12.98	18.92
Σ ω-3		45.53	18.51	16.99	32.07	33.50	29.16	29.74
Σ ω-6		15.43	10.13	11.78	17.97	16.84	17.96	16.61
ω-3:ω-6		2.95	1.83	1.44	1.78	1.99	1.62	1.79

Data are presented on a dry weight basis.
Highly unsaturated fatty acids (HUFAs): includes the PUFAs that contain 20 or more carbons.
ND = not detected.

semi-quantitatively scored based on the presence of supranuclear vacuoles, goblet cells, and inflammatory cell infiltrates (Table S1). Liver sections were semi-quantitatively scored for intrahepatic lipid and glycogen accumulation, cellular degeneration and necrosis, and inflammatory infiltrates as previously observed in this species when reared on alternative feeds (Rhodes et al., 2016). Both intestinal and liver sections were scored from 0 to 3 to indicate the severity of tissue alterations; 0 = no histologic changes observed; 1 = mild change; 2 = moderate change; 3 = diffuse change (Table S1).

2.4. Growth metrics and feed assessment

Sablefish growth and condition were measured for each treatment to determine the effects of the microalga-enriched fish-free feeds on sablefish. Percent weight gain (WG), specific growth rate (SGR), and thermal growth coefficient (TGC) were used to evaluate fish growth. Fulton's K and hepatosomatic index (HSI) were used to evaluate fish condition. Feed performance and nutrient utilization were assessed using dry feed intake (DFI), feed conversion ratio (FCR), protein efficiency ratio (PER), lipid productive value (LPV), and protein retention efficiency (PRE). The equations for each of these metrics can be found in the supplemental material.

2.5. Chemical analyses of tissues

The 18 initial sablefish euthanized before the experiment were prepared for whole-body proximate composition (three pooled samples of six fish each). Initial proximate composition and fatty acid methyl ester (FAME) analysis of sablefish skinned fillets could not be completed due to sample loss. After the experiment, five fish were sampled per tank and their homogenized tissue was pooled by tank (yielding three pooled samples per treatment). Each sample was then analyzed in duplicate for proximate composition. A single skinned fillet from four individual fish per tank were also pooled (yielding three pooled samples per treatment) and analyzed in duplicate for both proximate composition and fatty acid methyl ester (FAME) analysis. AOAC International (2019) methods were used to determine crude moisture (952.08), protein (990.03), fat (948.15), fiber (962.09), and ash (938.08) of sablefish whole-bodies (g/100 g wet weight). The same methods were used to determine the proximate composition of sablefish fillet samples, except for crude fat. Instead, total fat (total fatty acids) and the concentration of each fatty acid (expressed as % of total fatty acids) were determined (996.06). All sablefish samples were analyzed at NP Analytical Laboratories (St. Louis, Missouri, USA).

2.6. Data analysis

One-way analysis of variance (ANOVA) was used to test for significant differences in sablefish whole-body proximate constituents, fillet proximate constituents, individual fillet fatty acids, %WG, SGR, TGC, FCR, PER, LPV, PRE, Fulton's K, and HSI across treatments using the means from each tank ($n = 3$ tanks per treatment). Tukey's Honest Significant Difference test (Tukey's HSD) was used to determine significance levels between treatments if the ANOVA was significant. A linear regression analysis was used to determine if there was a linear relationship between microalga-inclusion (g/100 g) and sablefish growth metrics. Differences were considered significant when $p \leq 0.05$. Residuals were assessed for each analysis to ensure homoscedasticity assumptions were met. All statistical analyses were performed in R statistical software version 3.5.2 (R Core Team, 2018).

3. Results

3.1. Sablefish growth, condition, and feed utilization

Sablefish grew an average of 135% over the 140 days of this study from a mean starting weight of 334 g to a mean final weight of 783 g (Table 4). Initial mean sablefish weights were not different across treatments ($p = 0.207$). Sablefish growth, measured as percent weight gain (WG), specific growth rate (SGR), and thermal growth coefficient (TGC), was influenced by diet treatment (WG $p = 0.042$; SGR $p = 0.046$; TGC $p = 0.047$; Table 4). The Tukey post-hoc test did not determine which diet treatments were different for WG, SGR, or TGC; however, sablefish were largest and grew fastest in the +FM+FO, −FM+FO, and FF HighSc treatment groups and were smallest and grew slowest in the FF Flax and FF LowSc groups. In comparing the fish-free diet treatments, there was a positive linear relationship between sablefish SGR and inclusion of *Schizochytrium* sp. (Fig. 1; $y = 0.57 + 0.004(x)$, $r^2 = 0.35$, $p = 0.044$), with the highest SGR found in sablefish from the highest microalga-inclusion diet.

DFI was different across treatments ($p = 0.015$), with sablefish in the FO control (−FM+FO) group consuming the most feed, while sablefish in the FF LowSc group consumed the least (Table 4). LPV and Fulton's K were different across treatments (LPV $p = 0.046$; Fulton's K $p = 0.050$). While the Tukey post-hoc test did not identify which treatments contributed to this effect for either metric, LPV was highest in sablefish from the FF LowSc treatment and condition (Fulton's K) was highest in the fish-ingredient control treatment groups (+FM+FO and −FM+FO). There were no significant differences in FCR ($p = 0.165$), PER ($p = 0.347$), PRE ($p = 0.603$), or HSI ($p = 0.297$) across treatment groups (Table 4).

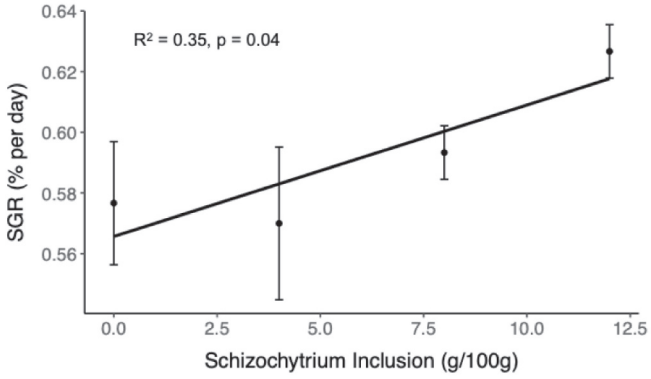

Fig. 1. Specific growth rate (SGR) of sablefish fed fish-free diets with increasing inclusion levels of the microagla, *Schizochytrium* sp. (0, 4, 8, and 12 g/100 g of the diet).

3.2. Fillet and whole-body proximate composition

Proximate analysis of sablefish whole-body samples (Table 5) indicated no significant differences for crude protein ($p = 0.821$), crude fat ($p = 0.166$), moisture ($p = 0.457$), or ash ($p = 0.063$) across diet treatments; however, ash content was only marginally non-significant and was lowest in the −FM+FO treatment. Sablefish mean whole-body crude protein was 14.6% and mean whole-body crude fat was 23.0%.

Sablefish fillet total fat (total fatty acids) was influenced by diet treatment ($p = 0.010$), such that sablefish fillets from the FF Flax treatment had higher total fat than sablefish fillets from the FF LowSc and FF HighSc treatment groups (Table 6; Tukey HSD $p < 0.05$). Sablefish fillet crude protein was not different across treatments ($p = 0.729$), and was 16.3% on average (1.7% higher on average than whole-body crude protein). Ash was higher in sablefish fillets from the FF LowSc and FF HighSc treatments than sablefish in the FF Flax treatment ($p = 0.009$; Tukey HSD $p < 0.05$). There was no difference in moisture across diet treatments ($p = 0.104$).

3.3. Histology of digestive tract

Histological semi-quantitative scoring of intestine tissue demonstrated no statistically significant differences between study groups for

Table 4
Growth, condition, and feed utilization metrics for sablefish.

	+FM+FO	−FM+FO	FF Flax	FF LowSc	FF ModSc	FF HighSc	*p*-value
IBW (g)	334 ± 2	334 ± 0	332 ± 1	335 ± 2	336 ± 2	331 ± 0	0.207
FBW (g)	811 ± 27	828 ± 21	746 ± 20	743 ± 32	774 ± 14	796 ± 11	0.092
WG (%)	142 ± 7	148 ± 6	125 ± 5	122 ± 8	130 ± 3	140 ± 3	0.042*
SGR (%/day)	0.63 ± 0.02	0.65 ± 0.02	0.58 ± 0.02	0.57 ± 0.03	0.60 ± 0.01	0.63 ± 0.01	0.046*
TGC	1.3 ± 0.05	1.37 ± 0.04	1.20 ± 0.04	1.18 ± 0.06	1.24 ± 0.03	1.31 ± 0.02	0.047*
Liver weight (g)	17.3 ± 0.8	17.8 ± 1.2	15.9 ± 1.0	15.7 ± 1.2	14.9 ± 0.6	15.8 ± 0.3	0.281
HSI	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	1.9 ± 0.0	2.0 ± 0.0	0.297
Fulton's K	1.03 ± 0.03	0.99 ± 0.02	0.99 ± 0.02	0.97 ± 0.00	0.95 ± 0.02	0.95 ± 0.01	0.050*
DFI (g/fish)	784 ± 30^{ab}	835 ± 30^{a}	717 ± 34^{ab}	653 ± 39^{b}	702 ± 16^{ab}	762 ± 27^{ab}	0.015*
FCR	1.64 ± 0.04	1.69 ± 0.07	1.73 ± 0.03	1.60 ± 0.02	1.59 ± 0.01	1.64 ± 0.03	0.165
PER	1.26 ± 0.03	1.26 ± 0.05	1.22 ± 0.02	1.31 ± 0.02	1.30 ± 0.01	1.26 ± 0.02	0.347
LPV	101.3 ± 10.9	120.1 ± 0.2	100.0 ± 4.5	125.6 ± 1.0	110.0 ± 3.3	114.7 ± 5.8	0.046*
PRE	19.2 ± 0.4	19.4 ± 0.6	17.2 ± 1.0	19.2 ± 0.4	18.7 ± 1.2	18.2 ± 0.8	0.603

IBW: initial body weight, FBW: final body weight, WG: weight gain, SGR: specific growth rate, TGC: thermal growth coefficient, HSI: hepatosomatic index, DFI: dry feed intake, FCR: feed conversion ratio, PER: protein efficiency ratio, LPV: lipid productive value, PRE: protein retention efficiency.
Data are presented as mean ± SE, $n = 3$ unless otherwise noted.
LPV and PRE are calculated with whole-body proximate values, $n = 2$ for +FM+FO, −FM+FO, FF LowSc, & FF HighSc treatments.
Values in the same row with different letter superscripts are significantly different.
Statistical significance is accepted at $p \leq 0.05$ and denoted by an asterisk*.

Table 5
Proximate composition of sablefish whole-body samples after 20 weeks fed treatment diets.

	+FM+FO $n = 2$	−FM+FO n = 2	FF Flax $n = 3$	FF LowSc n = 2	FF ModSc n = 3	FF HighSc n = 2	*p*-value
Crude Protein, %	15.1 ± 0.5	14.8 ± 0.1	14.3 ± 0.3	14.6 ± 0.0	14.5 ± 0.5	14.6 ± 0.1	0.821
Crude Fat, %	22.6 ± 1.3	24.5 ± 0.3	21.8 ± 0.5	23.1 ± 0.2	22.0 ± 0.4	23.9 ± 1.4	0.166
Moisture, %	61.3 ± 0.3	60.5 ± 0.2	61.7 ± 0.8	61.4 ± 0.1	62.2 ± 0.3	60.9 ± 1.1	0.457
Fiber, %	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	NA
Ash, %	1.98 ± 0.05	1.80 ± 0.05	1.94 ± 0.02	1.92 ± 0.04	1.97 ± 0.06	1.95 ± 0.02	0.063

Data are presented as mean ± SE; n = 3 for FF Flax and FF ModSc; n = 2 for +FM+FO, −FM+FO, FF LowSc, & FF HighSc.
Data are presented on a wet-weight basis.

Table 6
Proximate composition of fillet samples from sablefish fed treatment diets for 20 weeks.

	+FM+FO	−FM+FO	FF Flax	FF LowSc	FF ModSc	FF HighSc	*p*-value
Crude Protein, %	16.0 ± 0.3	16.9 ± 0.5	16.1 ± 1.2	15.7 ± 0.5	16.7 ± 0.4	16.5 ± 0.4	0.729
Total Fat, %	17.5 ± 0.7^{ab}	16.7 ± 0.9^{ab}	19.6 ± 0.4^{a}	14.3 ± 1.2^{b}	16.8 ± 0.9^{ab}	15.1 ± 0.5^{b}	0.010*
Moisture, %	64.2 ± 1.9	63.3 ± 0.9	63.0 ± 0.8	66.3 ± 0.5	65.4 ± 0.3	66.3 ± 0.5	0.104
Fiber, %	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	NA
Ash, %	1.19 ± 0.03^{ab}	1.20 ± 0.02^{ab}	1.13 ± 0.01^{b}	1.24 ± 0.01^{a}	1.19 ± 0.00^{ab}	1.23 ± 0.02^{a}	0.009*

Data are presented as mean ± SE, n = 3; Data are presented on a wet-weight basis.
Values in the same row with different letter superscripts are significantly different.
Statistical significance is accepted at $p \leq 0.05$ and denoted by an asterisk*.

the parameters examined (Table S2). Tissues appeared in good overall health with only minor histopathologic changes including low to moderate numbers of eosinophilic granular cells and fewer lymphocytes and plasma cells within the lamina propria and submucosa (Fig. 2a and 2b). Findings were consistent between experimental groups and interpreted to have minimal impact on fish health. Similarly, histological examination of liver sections (Fig. 2c and 2d) revealed no significant differences between study groups (Table S3). There was some variation in the amount of lipid/glycogen content within hepatocytes between individual fish but this was not associated with any one group. There were no observed changes associated with hepatocellular necrosis, nuclear pleomorphism, megalokaryosis, or regeneration, clear cell foci, stroma fibrosis, oval cell proliferation, or bile duct hyperplasia or epithelial vacuolation.

3.4. Fillet fatty acid composition

Individual fatty acid concentrations in sablefish fillets were different across diet treatments (Table 7). Fillet DHA (C22:6ω-3) concentration differed significantly as a function of the diet treatment ($p < 0.0001$).

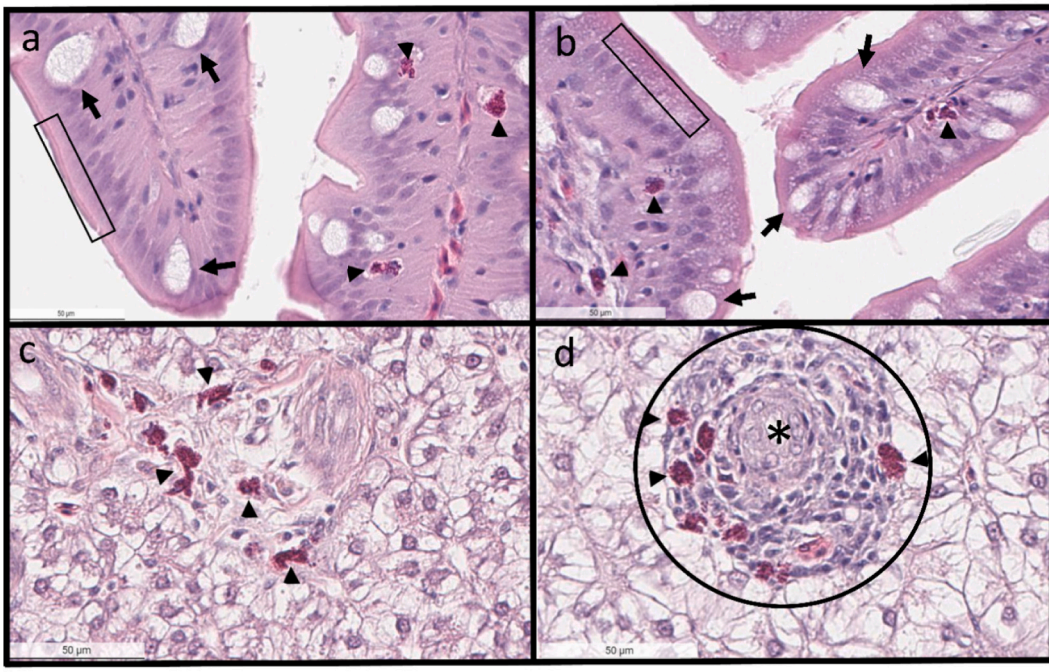

Fig. 2. Representative histopathology of intestine and liver of sablefish (*Anoplopoma fimbria*) in *Schizochytrium* sp. feed study. Intestinal villi (a and b) have low goblet cell density (arrows) and scattered eosinophilic granular cells (arrowheads). Supranuclear vacuoles within the apical portion of the epithelium are minimal (a) to low (b) (boxes). Hepatic sections (c and d) demonstrate mild eosinophilic granular cells infiltration (c) (arrowheads) and rare well-demarcated foci of mononuclear cells admixed with fewer eosinophilic granular cells (d) (circle) occasionally surrounding bile ducts (asterisk). Hepatocytes contain low (c) to moderate (d) amounts of lipid deposits.

Table 7
Sablefish fillet fatty acid composition (% total fatty acids) after 20 weeks fed treatment diets.

Fatty acid	Name	+FM+FO	−FM+FO	FF Flax	FF LowSc	FF ModSc	FF HighSc
Saturated fatty acids (SFAs)							
C14:0	Myristic acid	4.2 ± 0.1^{a}	3.8 ± 0.1^{a}	1.7 ± 0.7^{b}	1.8 ± 0.1^{b}	1.8 ± 0.0^{b}	1.9 ± 0.0^{b}
C15:0	Pentadecanoic acid	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.2 ± 0.0^{c}
C16:0	Palmitic acid	17.5 ± 0.1^{b}	17.4 ± 0.1^{b}	13.8 ± 0.0^{d}	15.4 ± 0.1^{c}	17.1 ± 0.0^{b}	18.1 ± 0.1^{a}
C17:0	Margaric acid	0.3 ± 0.0^{a}	0.3 ± 0.0^{b}	0.1 ± 0.0^{c}	0.2 ± 0.0^{c}	0.1 ± 0.0^{c}	0.2 ± 0.0^{c}
C18:0	Stearic acid	3.4 ± 0.0^{c}	3.5 ± 0.1^{c}	3.9 ± 0.0^{b}	4.2 ± 0.1^{a}	3.7 ± 0.1^{c}	3.6 ± 0.1^{c}
Monounsaturated fatty acids (MUFAs)							
C16:1ω-7	Palmitoleic acid	9.1 ± 0.1^{a}	8.7 ± 0.2^{a}	4.7 ± 0.0^{b}	4.8 ± 0.2^{b}	5.2 ± 0.2^{b}	5.2 ± 0.0^{b}
C18:1ω-7	Vaccenic acid	4.8 ± 0.1^{a}	4.7 ± 0.1^{a}	3.4 ± 0.1^{b}	3.2 ± 0.0^{b}	3.5 ± 0.1^{b}	3.3 ± 0.0^{b}
C18:1ω-9	Oleic acid	24.6 ± 0.7^{c}	25.8 ± 0.6^{bc}	30.2 ± 0.3^{a}	27.8 ± 0.5^{b}	27.4 ± 0.3^{b}	24.9 ± 0.3^{c}
Polyunsaturated fatty acids (PUFAs)							
C16:3ω-4	Hexadecatrienoic acid	0.72 ± 0.0^{a}	0.67 ± 0.0^{a}	0.25 ± 0.0^{b}	0.27 ± 0.0^{b}	0.25 ± 0.0^{b}	0.28 ± 0.0^{b}
C18:2ω-6	Linoleic acid	6.2 ± 0.1^{e}	7.5 ± 0.2^{d}	11.5 ± 0.1^{a}	10.3 ± 0.2^{b}	9.5 ± 0.2^{c}	7.9 ± 0.1^{d}
C18:3ω-3	a-Linolenic acid	2.5 ± 0.1^{e}	3.0 ± 0.1^{e}	18.4 ± 0.3^{a}	16.1 ± 0.4^{b}	10.3 ± 0.3^{c}	7.5 ± 0.1^{d}
C20:2ω-6	Eicosadienoic acid	0.46 ± 0.0^{a}	0.47 ± 0.0^{a}	0.42 ± 0.0^{b}	0.38 ± 0.0^{bc}	0.36 ± 0.0^{cd}	0.33 ± 0.0^{d}
C20:4ω-3	Eicosatetraenoic acid	0.89 ± 0.0^{a}	0.81 ± 0.0^{b}	0.32 ± 0.0^{e}	0.33 ± 0.0^{e}	0.40 ± 0.0^{d}	0.48 ± 0.0^{c}
C20:4ω-6	Arachidonic acid	0.9 ± 0.0^{a}	0.9 ± 0.0^{a}	0.5 ± 0.0^{d}	0.6 ± 0.0^{c}	0.6 ± 0.0^{c}	0.7 ± 0.0^{b}
C20:5ω-3 (EPA)	Eicosapentaenoic acid	6.3 ± 0.1^{a}	5.5 ± 0.1^{b}	2.2 ± 0.2^{c}	2.2 ± 0.2^{c}	2.3 ± 0.1^{c}	2.5 ± 0.1^{c}
C22:5ω-3	Clupanodonic acid	1.9 ± 0.0^{a}	1.7 ± 0.0^{a}	0.7 ± 0.1^{b}	0.7 ± 0.1^{b}	0.7 ± 0.0^{b}	0.8 ± 0.0^{b}
C22:5ω-6	Osbond acid	0.3 ± 0.0^{d}	0.3 ± 0.0^{de}	0.1 ± 0.0^{e}	1.2 ± 0.0^{c}	2.4 ± 0.0^{b}	3.4 ± 0.1^{a}
C22:6ω-3 (DHA)	Docosahexaenoic acid	5.4 ± 0.1^{c}	4.4 ± 0.2^{d}	1.7 ± 0.1^{e}	4.5 ± 0.2^{d}	8.2 ± 0.1^{b}	11.5 ± 0.2^{a}
Σ SFAs		26.2 ± 0.3^{a}	26.1 ± 0.7^{a}	19.5 ± 0.2^{d}	21.5 ± 0.3^{c}	22.7 ± 0.2^{bc}	23.7 ± 0.2^{b}
Σ MUFAs		39.9 ± 0.6^{a}	40.4 ± 0.7^{a}	38.5 ± 0.2^{ab}	36.3 ± 0.5^{c}	36.5 ± 0.3^{bc}	33.8 ± 0.2^{d}
Σ PUFAs		27.3 ± 0.3^{b}	26.9 ± 0.8^{b}	36.2 ± 0.1^{a}	36.7 ± 0.3^{a}	35.4 ± 0.4^{a}	36.0 ± 0.6^{a}
Σ HUFAs		17.7 ± 0.2^{b}	15.6 ± 0.4^{c}	7.3 ± 0.4^{e}	11.2 ± 0.5^{d}	16.2 ± 0.1^{bc}	21.0 ± 0.4^{a}
Σ ω-3 HUFAs		14.7 ± 0.2^{a}	12.7 ± 0.3^{b}	5.5 ± 0.3^{d}	8.2 ± 0.4^{c}	12.0 ± 0.1^{b}	15.5 ± 0.3^{a}
DHA + EPA		11.7 ± 0.2^{b}	9.8 ± 0.3^{c}	3.7 ± 0.3^{e}	6.7 ± 0.3^{d}	10.5 ± 0.1^{bc}	14.1 ± 0.3^{a}
Σ ω-3		18.0 ± 0.3^{c}	16.4 ± 0.5^{d}	23.5 ± 0.0^{ab}	24.2 ± 0.3^{a}	22.3 ± 0.3^{b}	23.2 ± 0.4^{ab}
Σ ω-6		8.0 ± 0.1^{c}	9.2 ± 0.1^{b}	12.3 ± 0.2^{a}	12.1 ± 0.2^{a}	12.8 ± 0.4^{a}	12.4 ± 0.1^{a}
ω-3 HUFA:ω-6		1.8 ± 0.0^{a}	1.4 ± 0.0^{b}	0.4 ± 0.0^{e}	0.7 ± 0.0^{d}	0.9 ± 0.0^{c}	1.2 ± 0.0^{b}
ω-3:ω-6		2.2 ± 0.0^{a}	1.8 ± 0.0^{c}	1.9 ± 0.0^{bc}	2.0 ± 0.1^{b}	1.8 ± 0.0^{c}	1.9 ± 0.0^{bc}

Data are presented as mean ± SE, $n = 3$.
Data were analyzed on a wet-weight basis.
Fatty acids with concentrations < 0.3% of total fatty acids for all treatments were not included in this table.
Highly unsaturated fatty acids (HUFAs): includes all PUFAs that contain 20 or more carbons.
All fatty acids listed were significantly different across diet treatments ($p < 0.0001$).
Values in the same row with different letter superscripts are significantly different (Tukey's HSD, $p < 0.05$).

DHA increased in fillets as microalga-inclusion increased in the diets (Fig. 3), with the highest fillet DHA concentrations in sablefish from the FF HighSc diet treatment (Tukey's HSD $p < 0.05$). EPA (C20:5ω-3) concentrations were higher in fillets from the fish-ingredient control groupscompared to fillets from the fish-free treatment groups (Fig. 3; $p < 0.0001$; Tukey's HSD $p < 0.05$).

Summed saturated fatty acid (SFA) concentrations in fillets (Table 7) were highest in sablefish from the +FM+FO and −FM+FO treatments, and lowest in fillets from the FF Flax treatment group ($p < 0.0001$; Tukey's HSD $p < 0.05$). Palmitic acid (C16:0) was the most prevalent SFA in all six diets. Sablefish fillets from the FF HighSc treatment had the highest concentrations of palmitic acid, followed by the +FM+FO, −FM+FO, and FF ModSc treatment groups, with the lowest concentration in fillets from the FF Flax treatment ($p < 0.0001$; Tukey's HSD $p < 0.05$). The sum of the monounsaturated fatty acids (MUFAs) in fillets was highest in sablefish fillets from the +FM+FO and −FM+FO

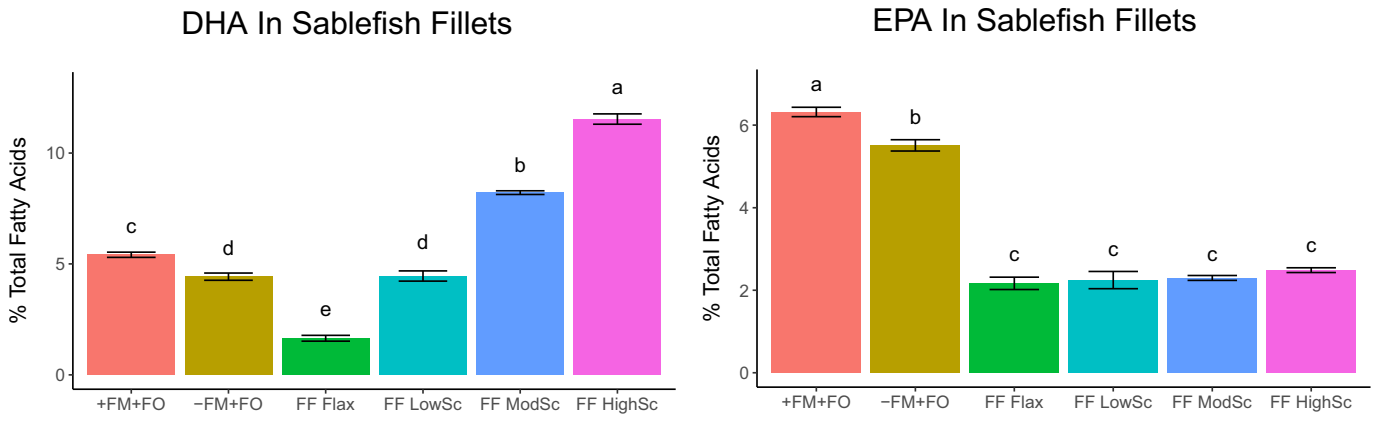

Fig. 3. DHA and EPA (% of total fatty acids) in sablefish fillets for each diet treatment group. Data are presented as mean ± SE (n = 3).

treatments and the lowest in sablefish from the FF HighSc treatment group ($p < 0.0001$; Tukey's HSD $p < 0.05$).

Summed PUFA concentrations in sablefish fillets (Table 7) were higher in the fish-free diet treatments than in the fish-ingredient control treatments, which were not different from each other ($p < 0.0001$; Tukey's HSD $p < 0.05$). The largest contributors to the omega-3 PUFAs in the fillets of fish-free treatment fish were α-linoleic acid (C18:3ω-3) and DHA (C22:6ω-3). Summed fillet HUFA concentrations did not reflect the same trend as overall fillet PUFA concentrations (Table 7). Summed HUFA concentrations (including ω-3 and ω-6 HUFAs) in fillets were highest in sablefish fed the FF HighSc diet, while sablefish fed the FF ModSc diet had fillet HUFA values equal to the +FM+FO control ($p < 0.0001$; Tukey's HSD $p < 0.05$). When looking at the subset of ω-3 HUFAs, sablefish from the +FM+FO and FF HighSc treatments had higher fillet ω-3 HUFA concentrations than all other treatments ($p < 0.0001$; Tukey's HSD $p < 0.05$). Fillet omega-3 to omega-6 ratios ranged from 1.9 to 2.2 (Table 7), and was highest in sablefish from the +FM+FO diet treatment, while fillet ω-3:ω-6 ratios from the −FM+FO control were not different from the FF HighSc and FF ModSc treatment groups (Fig. 4; $p < 0.0001$; Tukey's HSD $p < 0.05$). The ratio of ω-3 HUFAs to total ω-6 fatty acids ranged from 0.4 (FF Flax) to 1.8 (+FM+FO), with the −FM+FO and FF HighSc diets yielding the second highest fillet ω-3 HUFA:ω-6, and the FF Flax diet yielding the lowest ω-3 HUFA:ω-6 (Table 7; Fig. 4).

4. Discussion

In light of the current variable and reduced landings of wild-caught forage fish used for fish meal (FM) and fish oil (FO) production and the projected expansion of fish aquaculture, successful commercial culture will require identification of alternative feed ingredients that fulfill nutrient requirements for the cultured species, while maintaining nutrition profiles expected by human consumers. This study investigated a combination of *Schizochytrium* sp. and flax oil to completely remove FO while maintaining high levels of omega-3 fatty acids in the feeds. Though feed studies are often conducted with small juvenile fish (5 g - 100 g initial weight), the current study used sablefish with an initial mean weight of 334 g and a final mean weight of 783 g. Since nutrient requirements of fish change with age, studying larger fish in feed trials is necessary in order to understand how these grow-out sized sablefish will perform and utilize nutrients in feeds at an industry-relevant size, which is when the majority of the feed is utilized in aquaculture.

4.1. Sablefish growth, condition, nutrient utilization, and histology

There was a dietary effect on sablefish growth. Sablefish fed the high microalga-inclusion diet (FF HighSc) and both fish-ingredient controls (+FM+FO and −FM+FO) had mean SGRs ≥ 0.63% per day, while sablefish fed all other fish-free treatment diets had mean SGRs ≤ 0.60% per day. This suggests that the highest inclusion level of *Schizochytrium* sp. (12 g/100 g) is beneficial to sablefish growth when fed a fish-free feed, which is likely due to the higher HUFA concentrations compared to the other fish-free diets. Growth results from this study are consistent with results from Goetz et al. (2021) where juvenile sablefish fed an alternative flax oil feed containing 3.5 g/100 g DHASCO microalgae oil (Martek Biosciences, Columbia MD, USA) grew at a similar rate to those fed the same diet with fish oil. This is also in support of what has previously been seen in juvenile Nile tilapia when fish oil was completely replaced with *Schizochytrium* sp. (Sarker et al., 2016). While there are no optimized diets for sub-adult sablefish, in the current study sablefish had comparable growth to what was reported by Friesen et al. (2013b) for a similar size class of sablefish. Notably, dietary lipid content in the present study was 15%, which is considerably lower than the 19% dietary lipid used by Friesen et al. (2013b), suggesting adequate dietary lipid inclusion for this size class may be lower than previously demonstrated.

To assess the efficiency of the test diets for sablefish growth, one of the metrics used was feed conversion ratio (FCR). It has been documented that FCR increases (lower feed efficiency) as fish get larger and growth slows. Reid et al. (2017) reported FCRs for sablefish between 1.34 (920 g mean final weight) and 1.62 (final weight > 920 g). The sub-adult sablefish in the current study (783 g final mean weight) had a FCR of 1.65 on average over the course of the trial, which is higher than expected for sablefish of this size based on the available information. This means the sablefish in the current study converted feed into body mass less efficiently than what was reported by Reid et al. (2017). However, a FCR of 1.65 is in line with the mean FCRs reported for other commonly farmed fish species like carp, catfish, and trout, yet still higher than Atlantic salmon (Fry et al., 2018). FCR is widely used in feed studies, but it is an imperfect measure of feed efficiency across species and diets. While FCR does give some indication as to the utilization of a feed, it does not account for differences in protein and lipid content.

For a more specific examination of nutrient utilization, protein retention efficiency (PRE) was used, which is a measure of protein gain based on protein consumed. Sablefish in this study had a mean PRE of 18.5%, which was not different across treatments. Nicklason et al. (2020) reported PREs of 22-24% when sablefish were smaller (245 g mean final weight) and Johnson et al. (2020) observed PREs of 27-30% when sablefish were smaller still (170 g mean final weight) and consuming a feed with similar dietary protein (46-48%). This trend suggests that PRE declines as sablefish get larger, which is consistent with what has been observed for other growth and feed conversion parameters in fish (Árnason et al., 2009; Björnsson et al., 2001). However, it is important to note PRE is also a reflection of amino acid balance in the feed and quality of the protein. While this is known, the amino acid compositions of the diets were not analyzed for the current study.

Lipid productive value (LPV) is a measure of whole-body lipid gain based on lipid consumed. While the Tukey post-hoc test did not identify which diet treatments were significantly different, sablefish in the FF

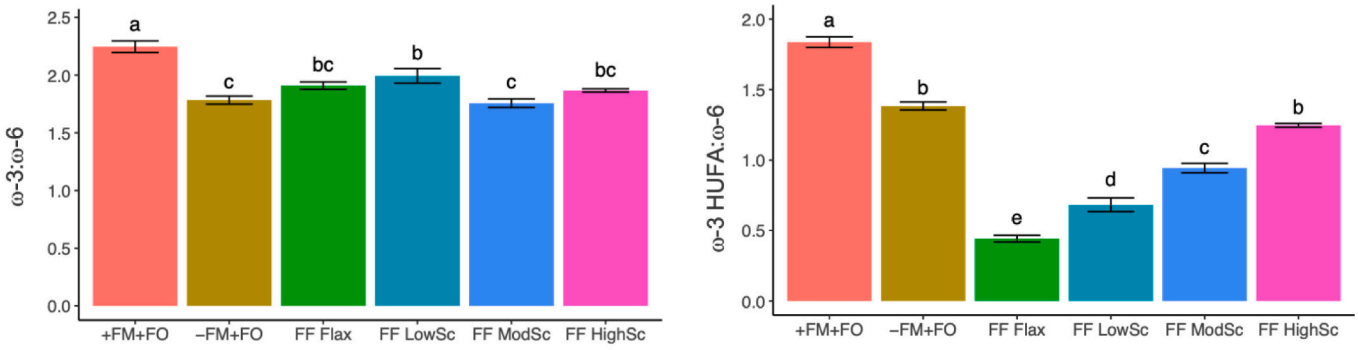

Fig. 4. Omega-3 to omega-6 ratio (ω-3:ω-6) and ω-3 HUFA:ω-6 for sablefish fillets of each treatment group. Data are presented as mean ± SE (n = 3).

LowSc treatment had the highest LPV. Sablefish in this treatment also had the lowest DFI, yet the proximate analysis results for whole-body crude fat were not different across treatments. This indicates that sablefish are likely very efficient with dietary lipid incorporation, which is not completely unexpected since sablefish are commonly called butterfish and are well-known for their high oil content. Proximate analysis of whole-body samples showed crude fat was 23.0%. This fat content is higher than wild-caught sablefish, but is consistent with what has been reported for sablefish reared in a laboratory setting (Sogard and Spencer, 2004). Additionally, whole-body crude fat of 23% is similar to what was reported for sablefish by Friesen et al. (2013b) when dietary lipid was higher at 19%, further supporting the supposition that sablefish can efficiently utilize dietary lipid when provided at lower levels in the feeds.

Intestine and liver sections were examined histologically to assess the impact of *Schizochytrium* sp. on intestinal and hepatic morphology. Pathologic changes have been described in these tissues secondary to alternative diets that were interpreted to be poorly tolerated. Diets that are overly antigenic and produce metabolic stress have been associated with extensive enteritis, loss of intestinal mucosal integrity, increased goblet cell density and decreased supranuclear vacuoles (Wang et al., 2017; Knudsen et al., 2008), while liver sections can display hepatic steatosis (Roberts and Ellis, 2012) and hepatocellular degeneration and necrosis (Chaklader et al., 2020). No abnormal cellular alterations were present in histologic evaluation of intestine and liver of sablefish fed the microalga-inclusion diets, and histomorphologic findings were not different than fish fed the control diets. There was mild to moderate infiltration of eosinophilic granular cells and lymphocytes and plasma cells within the connective tissue of the intestine and liver in all fish. This level of inflammatory infiltrates is commonly observed in healthy fish and consistent with non-specific antigenic stimulation with minimal effect on fish health (Oztop, 2023). Hepatic cells contained low to moderate amounts of glycogen and lipid deposition with minor variation between fish and no significant differences between treatment groups. This is consistent with adequate nutrient storage and positive energy balance (Yani et al., 2015; Rhodes et al., 2016). These results indicate that the micro-algal inclusion diets were tolerated as well as the control diet and consistent with healthy intestinal and hepatic histomorphology.

4.2. Fillet fatty acid composition

Fatty acid concentrations in sablefish fillets differed across diet treatments. While all four fish-free treatments resulted in higher PUFAs than the fish ingredient controls, the composition of these PUFAs varied greatly. Sablefish fillets from the FF Flax treatment contained the highest linoleic acid and α-linoleic acid concentrations and the lowest total HUFA concentration compared to all other diets. Sablefish fillet HUFA concentrations (mainly EPA and DHA) increased as dietary microalga inclusion increased. The Academy of Nutrition and Dietetics recommend approximately 250-500 mg per day, or 1.75-3.5 g per week of combined DHA + EPA for humans (Vannice and Rasmussen, 2014). A single 100 g fillet from sablefish in the FF ModSc and FF HighSc treatments would contain the weekly recommended amount of combined DHA + EPA (1.77 g and 2.14 g, respectively), which is similar to the combined DHA + EPA in fillets from sablefish fed the +FM+FO reference diet (2.04 g/100 g fillet). This indicates that the higher inclusion levels of *Schizochytrium* sp. in this study adequately supplied DHA for sablefish when FO was removed from the diets. These values are similar to wild-caught Atlantic herring (2.2 g/100 g) and higher than farmed Atlantic salmon (1.2 g/100 g) (Nøstbakken et al., 2021).

While HUFAs are important, HUFA concentration alone is not sufficient for an accurate assessment of fillet fatty acid composition since the ratio of omega-3 to omega-6 fatty acids have important health implications (Simopoulos, 2002). The range of fillet ω-3:ω-6 ratios across fish species is quite large, from 1.2 for wild-caught catfish to 6.0 for wild-caught rainbow trout (Nettleton, 2001). When culturing seafood species, it is common for the cultured fish to have a relatively lower ω-3:ω-6 ratio than their wild counterparts (Nettleton, 2001) due to diet differences; however, a higher ω-3:ω-6 ratio is preferential. In the present study, sablefish fillet ω-3:ω-6 ratios ranged from 1.8 to 2.2, compared to previously reported ratios of 1.4 for farmed sablefish (Watters et al., 2012) and 5.4 for wild sablefish (Cladis et al., 2014). Sablefish fed the +FM+FO control diet, which had the lowest amount of terrestrial ingredients, had fillets with the highest ω-3:ω-6 ratio. The addition of terrestrial ingredients typically increases ω-6 content, which decreases ω-3:ω-6 ratios. Poultry fat, soybean oil, and corn oil all have ω-3:ω-6 ratios under 0.1 (National Research Council, 2011). Flax oil is slightly better with a ω-3:ω-6 ratio of 4.2, but this is still much less than the ω-3:ω-6 ratios of FO from common pelagic fish species that are typically in excess of 12.

Notably, in this study fillet ω-3:ω-6 ratios did not differ markedly between treatments (Fig. 4) despite the differences in marine ingredient inclusion levels, which are known to contain higher ω-3:ω-6 ratios. This is especially interesting when considering the FF Flax treatment (fish-free, terrestrially sourced ingredients), which produced sablefish fillets with the same ω-3:ω-6 ratio as the microalga-inclusion treatments and the FO control (−FM+FO). This observation alone suggests the microalga may not be required to produce comparable ω-3:ω-6 ratios to the FO control; however, the ω-3 fatty acids present in sablefish fillets from the FF Flax treatment were short-chain omega-3 PUFAs. In order to detect the contribution of the long-chain ω-3 HUFAs to the ω-3:ω-6 ratio, it is important to consider the ratio of ω-3 HUFAs to total ω-6 fatty acids. The FF HighSc treatment and the FO control produced fillets with the same ω-3 HUFA:ω-6 ratios (Fig. 4), demonstrating the effectiveness of this microalga as a FO replacement that maintains similar beneficial HUFA concentrations. These are important human nutritional health considerations when evaluating alternative lipids for seafood production.

DHA and EPA are considered essential fatty acids for fish, derived from dietary sources including microalgae. However, there is evidence that retroconversion of DHA to EPA occurs in some terrestrial vertebrates (Hagve and Christophersen, 1986; Park et al., 2016), but this has not yet been studied in fish. This of interest due to the nature of current commercially available microalgae products, which typically contain high concentrations of DHA and low concentrations of EPA. In this study, there was no evidence of a relationship between fillet EPA concentration and diet treatment from the fish-free treatments, indicating that retroconversion of DHA to EPA did not occur in sablefish over the 20-week period. However, fillet EPA concentrations were higher than feed EPA concentrations in sablefish from the microalga-inclusion diet groups. The concentration of EPA in the fish-free feeds ranged from 0.63-1.18% of total fatty acids, yet the EPA concentrations in the fillets were 2.3% on average for those four treatment groups (Fig. 5). One

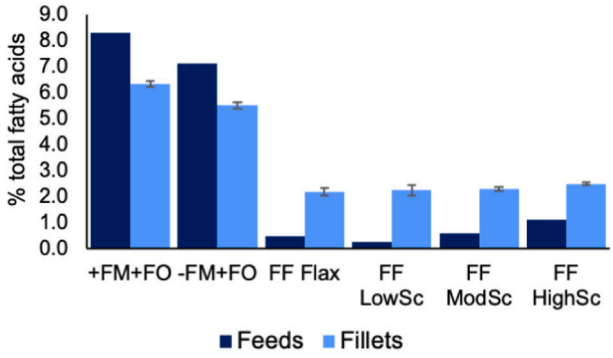

Fig. 5. The concentration of EPA (% of total fatty acids) in the treatment feeds (dark blue bars) and in sablefish fillets (light blue bars) for each treatment. Fillet data are presented as treatment mean ± SE (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

possibility for this is that the sablefish retained some of the EPA they acquired before the start of the experiment. This could be due to the "omega-3 sparing effect", which has been documented in other marine fishes (Emery et al., 2016; Turchini et al., 2011). The availability of SFAs and MUFAs in the diet may provide the fish with sufficient fats for energy metabolism, permitting retention of ω-3 HUFAs in the body, sparing these essential fatty acids for other vital physiological processes (Emery et al., 2016). This indicates sablefish may not need EPA in their diets at high levels after the juvenile stage. An extended study would need to be conducted to determine how long sablefish can retain certain fatty acids when they are not provided significantly in the diet and if any adverse effects are expressed later.

5. Conclusion

The results of this study indicate that sablefish growth, condition, and fillet proximate composition are not negatively affected by the complete replacement of FM and FO. The combination of dried whole-cell *Schizochytrium* sp. and flax oil can be used to replace FO in sablefish feeds, with higher inclusion of the microalga resulting in higher fillet HUFA concentrations. While the cost of microalgae may currently be higher than terrestrial lipid alternatives, there are inherent biological benefits when omega-3 HUFAs are provided in the diets that are otherwise lacking in most terrestrial alternatives. Additionally, the cost of ingredients is linked to the scale of production; therefore, widescale adoption of novel ingredients in aquaculture can reduce costs. The replacement of FM and FO in aquaculture feeds will greatly reduce pressure on wild forage fish stocks and allow for a more sustainable expansion of the aquaculture industry. While additional feed studies would be beneficial for improving the efficiency of sablefish production on fish-free feeds, this study shows it is biologically feasible to completely replace FO with microalgae and flax oil in sablefish feeds without significantly impacting growth, all while maintaining nutritionally beneficial fillet fatty acid profiles for human consumers.

Statement of author contribution

KAN designed the study, performed fish husbandry, completed data analyses, and wrote the manuscript. LDG contributed to study design, sampling, and analyses. RBJ and FTB contributed to study design and logistics. SLH contributed to study design and analyses. FTB formulated the experimental feeds. DPM completed histomorphologic assessments. All authors contributed to the manuscript.

Declaration of Competing Interest

The authors have no known financial or personal interests that would influence the work reported in this study.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank Matt Hoehn, Andrew Kim, and Kevin Hinterman at MLML for assisting with fish husbandry and system maintenance throughout the experiment. The authors also thank Rick Goetz and his team at NOAA Fisheries Manchester Research Station for the production and gift of the sablefish for this study. Thank you to Grant Gilman at Star Milling for donating the microalga for the feeds. This study was funded by California Sea Grant through the Graduate Research Fellowship Program (Grant # R/AQ-158F).

Appendix A. Supplementary data

Supplementary material for this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.740000.

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