

Elucidating an optimal diet for captive *Acropora* corals

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ARTICLE INFO

Keywords:

Coral
Nutrition
Lipids
Acropora
Fatty acids

ABSTRACT

Developing an optimal heterotrophic feeding regime has the potential to improve the growth and overall health of captive corals. This study evaluated the efficacy of seven unique feeding strategies for *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* nubbins: a novel, micro-bound diet (ATF), a novel, dissolved diet rich in long-chain polyunsaturated fatty acids (LC-PUFA) (LONG), a novel, dissolved diet lacking in LC-PUFA (SHORT), a phytoplankton diet of *Isochrysis galbana* (ALG), co-feeding of LONG and ALG (COMB), unfiltered seawater (RAW), and an unfed treatment of filtered seawater only (CTL). After 90 days, all species showed the highest growth in the ALG treatment. There was a negative correlation between growth and total lipid concentration, indicating that *Acropora* corals have a low tolerance for lipid-rich diets. Coral appeared to perform best in the relatively natural treatments, namely the ALG, CTL, and RAW, which recorded higher growth and increased proportions of high-energy lipid compounds such as wax esters, triacylglycerols, and the fatty acids, 16:0 and 22:6n-3. Meanwhile the formulated diets (ATF, LONG, SHORT) promoted greater proportions of structural lipids and indispensable, membrane-bound fatty acids including 20:5n-3 and 20:4n-6. Despite the SHORT diet being low in LC-PUFA, corals fed this diet showed few significant differences in several individual LC-PUFA compared to most other treatments, suggesting that these were obtained *via de novo* synthesis, or selectively retained from initial reserves. Ultimately, this study showed that phytoplankton represents a promising vehicle of nutrient delivery for captive *Acropora* corals.

1. Introduction

The decision to grow a new organism in a large-scale aquaculture system requires evaluation of a great and disparate number of considerations. Of particular importance are the biological criteria that lend a species the necessary characteristics to be domesticated. Whilst numerous coral species have been assessed for their captive culture potential, scleractinian corals of the family Acroporidae represent promising candidates for aquaculture owing to their comparatively fast growth, high fecundity, and relative abundance on reefs (Knop and Moorhead 2013; Williams et al., 2013). Additionally, *Acropora* sp. are highly popular organisms in the aquarium trade (Knop and Moorhead 2013) and ecologically important in the reef ecosystem (Hemond et al., 2014; Pollock et al., 2017). As such, *Acropora* corals represent promising candidates for captive rearing, and advancing their aquaculture prospects through optimised heterotrophic feeding regimes is of significant economic and environmental interest. Increased heterotrophic feeding has been shown to be highly beneficial to corals, promoting increased organic matrix secretion, calcification and photosynthesis

rates, as well as concentrations of chlorophyll *a*, lipids and protein (Houlbrèque and Ferrier-Pagès, 2009; Towle, 2015).

In order to understand the physical and biochemical characteristics required to create a readily accepted and nutritionally appropriate diet for *Acropora* corals in captivity, it is necessary to consider the various feeding modes employed by this genus. Although the majority of the coral host's daily energy requirements are satisfied by photosynthesis (40–95%) (Falkowski et al. 1993; Titlyanov and Titlyanova 2002; Mieog et al. 2009), scleractinian corals are also highly opportunistic heterotrophic feeders, exploiting several trophic pathways simultaneously (Nahon et al., 2013). In the wild, scleractinians are known to span the levels of primary producer, herbivore, carnivore, detritivore and consumer of dissolved nutrients (Houlbrèque and Ferrier-Pagès, 2009; Nahon et al., 2013). These exogenous feeding modes provide corals with important nutrients such as nitrogen, phosphorus, and alternative forms of lipids that are not provided by photosynthesis (Houlbrèque and Ferrier-Pagès, 2009).

With regard to captive coral feeding, two recent works showed that, of a range of different food options, *Acropora* corals performed best

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<https://doi.org/10.1016/j.aquaculture.2019.734420>

Received 29 April 2019; Received in revised form 21 August 2019; Accepted 21 August 2019

Available online 24 August 2019

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when provided with unfiltered seawater (Conlan et al., 2018, 2017a). This is likely attributable to the diverse feeding habits of this genus, since unfiltered, 'raw' seawater constitutes a natural source of dissolved nutrients, suspended particulate matter (SPM), and microbiota (Fabricius, 2005). Despite this, the potential for bacteria and pathogen introduction, the high infrastructure costs of natural seawater access, and seasonal fluctuations in salinity and nutrient loads render unfiltered, 'raw' seawater unfeasible for large-scale coral culture. As such, further investigation is required to elucidate a dietary vehicle that can achieve growth and health levels akin to those possible with natural feeding regimes.

Examination of the unfiltered seawater tested by Conlan et al. (2017a) and Conlan et al. (2018) showed high chlorophyll *a* concentrations. This indicates the presence of phytoplankton (Ojea et al., 2004) and therefore suggests herbivory may be employed as a feeding mode by *Acropora* corals. Although only a limited number of studies have investigated herbivory in scleractinian corals (Leal et al., 2014; Seemann et al., 2013), phytoplankton presents a highly nutritious food source (Piccinetti et al., 2016) and consumption has been recorded in numerous other coral taxa (Fabricius et al., 1995; Gomes et al., 2012; Orejas et al., 2016). Furthermore, *Acropora* corals have been shown to have a lower capacity to capture live prey compared to other coral species with similar polyp sizes (Hoogenboom et al., 2015), suggesting that this genus may be more adept at capturing smaller, less motile prey such as phytoplankton.

Acropora corals have also been shown to readily consume SPM (Anthony, 1999; Bythell, 1988; Towle et al., 2015). In coral reef environments, SPM constitutes a diverse food item that often becomes colonised by microorganisms and coated with organic nutrients, increasing its nutritional value (Anthony, 1999; Wotton, 1994). Moreover, SPM provides a concentrated nitrogen (N) and phosphorus (P) source (Anthony, 1999), rendering it a potentially important nutrient delivery vehicle for corals. However, an artificial, micro-bound diet resembling the characteristics of SPM examined by Conlan et al. (2018, 2017a) in *Acropora* sp. recruits and *A. millepora* nubbins yielded poor results, including lower survival, growth and overall health compared to unfiltered seawater. This may be explained by poor digestibility, a low tolerance for nutrient binding compounds, or a greater preference for captive *Acropora* corals to receive nutrients in the dissolved form (Anthony, 1999; Bythell, 1990).

Previous studies have shown that the uptake of nitrate and ammonium from natural seawater contributes approximately 30% of the daily N requirement in captive *Acropora palmata* (Bythell, 1990, 1988), while dissolved phosphate was shown to promote increased growth rates in captive *Acropora muricata* (Dunn et al., 2012). Furthermore, slightly elevated dissolved N and P concentrations have also shown increased growth and photosynthetic efficiency in numerous other coral species (Dunn et al., 2012; Fabricius, 2005; Godinot et al., 2011). As such, further investigation regarding the importance of dissolved nutrients as a dietary vehicle in coral nutrition is warranted.

In addition to optimising the delivery mode of captive coral diets, the nutritional profile must also be formulated to meet physiological requirements. In particular, understanding the dietary fatty acid (FA) requirement is an important consideration in coral nutrition. FA provide metabolic energy for coral growth and reproduction and are required for a suite of essential metabolic processes such as; cellular lipid synthesis, lipid turnover, eicosanoid production, and membrane fluidity (Conlan et al., 2017b and references therein). Numerous studies have also demonstrated the dietary importance of long-chain polyunsaturated fatty acids (LC-PUFA) in corals, particularly, concerning their involvement in stress resistance and recovery (Lim et al., 2017; Seemann et al., 2013; Tagliafico et al., 2017). Specifically, an appropriate balance of the individual LC-PUFA, 20:5n-3 (EPA), 22:6n-3 (DHA), and 20:4n-6 (ARA) is required for optimal biochemical and physiological functioning, cell membrane structure and metabolic energy in marine organisms (Brett and Muller-Navara, 1997; Tocher,

2003).

Dietary FA, including, EPA, DHA, and ARA are generally considered essential given the inability of most animals to synthesise these FA *de novo*. However, the presence of endosymbionts of the Symbiodiniaceae family (henceforth referred to as symbionts) and other endogenous microbiota within the coral holobiont shroud the origin of several LC-PUFA (Imbs et al., 2009; Monroig et al., 2013). This is particularly relevant when considering some symbiont clades are able to synthesise LC-PUFA from shorter chain homologues (Imbs et al., 2010). As such, the dietary inclusion of some LC-PUFA may be superfluous to physiological requirement.

Therefore, in an attempt to satisfy the requirements for exogenous nutrient ingestion and assimilation in captive corals, the present study evaluated the efficacy of a variety of feeding strategies (phytoplankton, bound nutrient particles, and dissolved nutrients) in three *Acropora* species: *A. loripes*, *A. millepora*, and *A. tenuis*. Additionally, in order to assess the efficacy of feeding a diet rich in LC-PUFA to captive *Acropora* corals, the present study examined an LC-PUFA-rich diet against an LC-PUFA-deficient diet. Since corals are generally opportunistic, passive feeders, whose exogenous intake may be highest at night (Houlbrèque and Ferrier-Pagès, 2009), all diets were fed continuously, to ensure maximum opportunity for consumption. After 90 days, the effects of each treatment were determined through an assessment of growth, total protein, total lipid and its constituent classes and FA.

2. Materials and methods

2.1. Colony collection and experimental design

Colonies of *A. millepora*, *A. loripes*, and *A. tenuis* were collected from Davies Reef, Queensland, Australia (lat.: 18°83.162'S, long.: 147°63.45'E) between the 20th and 24th of March, 2015. Corals were transferred to the National Sea Simulator facility at The Australian Institute of Marine Science (AIMS, Townsville, Australia, lat.: 16°17.728'S, long.: 145°27.121'E). A total of 900 nubbins (300 nubbins species⁻¹) were prepared by cutting the apical branches off the colonies and gluing them to aragonite plugs (Ocean Wonders®). Initial nubbin weights ranged from 6 to 11 g. The nubbins were acclimated for two weeks, by which time tissue had entirely covered the aggrieved skeleton. During this period, nubbins were fed once daily with enriched rotifers (*Brachionus plicatilis*, enriched for 24 h with INVE Selco® S.parkle) and tanks were maintained in a partially re-circulated system, under an irradiance of 290 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (illumination period: 9.5 h day⁻¹, 2 h ramp time, favouring blue light), and temperature of 26.8 $\pm$ 0.2 °C. Water movement in each aquaria was provided by Tunze® Turbelle® nanostream® 6045 circulation pumps providing water flow of $\sim$ 10 cm sec⁻¹.

Following acclimation, nubbins were equally divided into 21 49-L tanks with flow-through seawater supply (5 nubbins species⁻¹ tank⁻¹). Nubbins were maintained under identical conditions with a flow-through rate of 0.8 L min⁻¹ (1 turnover hour⁻¹) and a circulation pump (Tunze® Turbelle® nanostream® 6015) provided water flow of $\sim$ 10 cm sec⁻¹. Temperature and light intensity remained constant at 26.8 °C and $\sim$ 290 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (illumination period: 12 h day⁻¹, 2 h ramp time, favouring blue light), respectively. Three replicate tanks were randomly assigned one of seven feeding regimes in a single-factorial design:

1. Control (CTL): Flow-through ultra-filtered seawater (0.04 μm) with no additives.
2. Unfiltered (RAW): Unfiltered seawater sourced from Cleveland Bay (lat.: 19°155.83'S, long.: 146°88.116'E) subjected to a hydro-cyclone filtration only.
3. Artificial (ATF): A LC-PUFA-rich, sodium alginate-bound formulated diet developed at AIMS. (3 g tank⁻¹ WW). This diet contained a mixture of intact and hydrolysed marine and terrestrial ingredients.

(Commercial in confidence formulation, details not provided). This diet was of the same formulation as those described in Conlan et al. (2018, 2017a). In the present study, this diet was provided continuously in an attempt to improve its efficacy.

4. LC-PUFA-rich diet (LONG): A LC-PUFA-rich, unbound, formulated diet developed at AIMS. (3 g tank⁻¹ WW)
5. LC-PUFA-deficient diet (SHORT): A formulated, unbound diet developed at AIMS, containing comparatively low LC-PUFA levels. (3 g tank⁻¹ WW)
6. Phytoplankton (ALG): *Isochrysis galbana* cultured at AIMS using F2 culture media (1000 cells mL⁻¹) (*I. galbana* was chosen due to its naturally high levels of DHA (Olsen et al., 2000)).
7. Combination (COMB). Co-feeding of the ALG and LONG diets in equal parts. (500 cells mL⁻¹ and 1.5 g tank⁻¹ WW, respectively).

The two LC-PUFA-rich formulated diets (ATF and LONG), were identical in their formulation with respect to specific ingredients. These diets differed only in the presence or absence of a sodium alginate binder. With the exception of the RAW treatment, all other treatment tanks were provided with CTL seawater.

2.2. Feeding and husbandry

Since corals are generally opportunistic, passive feeders, whose exogenous intake may be highest at night (Houlbrèque and Ferrier-Pagès, 2009), all diets were fed continuously, to ensure maximum opportunity for consumption. All feeding treatments were compared against a comparatively natural feeding treatment of unfiltered seawater, as well as an unfed and wholly phototrophic treatment of ultrafiltered seawater. All formulated diets were prepared fresh daily. *I. galbana* was harvested daily and the cell density confirmed under light microscopy using a haemocytometer. All diets were diluted to 4 L in ultrafiltered seawater. Diets were administered to experimental tanks continuously through a peristaltic pump at 2.8 mL min⁻¹. This equated to 2.1 mg min⁻¹ for the formulated diets (3 g day⁻¹), and 2800 cells min⁻¹ for the ALG diet (4 × 10⁶ cells day⁻¹) (dosage based on preliminary flow cytometry results of RAW seawater). The continuous input of the exogenous diets, as well as the inclusion of water circulation pumps in each tank, ensured that each diet was always available in the water column, regardless of being alive or inert.

Formulated diets (ATF, LONG, SHORT, and COMB) were kept refrigerated throughout and phytoplankton diets (ALG, COMB) were kept at room temperature under a 12:12 h light regime at ~300 μmol photons m⁻² s⁻¹. For the COMB treatment, the two dietary components were stored in separate reservoirs and combined within the feed line. All reservoirs contained air stones to maintain homogeneity and aerate the phytoplankton. Feed lines were rinsed twice daily with hot, soapy water for 10 min to remove diet residue, following by double rinsing with fresh water. Tanks were siphoned daily to remove remaining food and detritus, while twenty herbivorous snails (*Thalotia strigata*) were included in each tank to assist with algae and microfilm control from tank walls.

2.3. Coral sampling

Experimental duration was 90 days. Prior to commencement, seven coral nubbins from each colony were sampled for initial biometrics and nutritional profiling (Supplementary Tables S1 & S2). All experimental nubbins were initially weighed (to nearest 0.001 g) using the buoyant weight method (Davies, 1989). After 90 days, all experimental nubbins were analysed for buoyant weight, proximate composition, lipid class, and FA composition. Due to the presence of large quantities of organic material in coral skeletons, all coral nubbins were freeze-dried and crushed *in toto* prior to analyses according to the method described by Conlan et al. (2017a, 2017b).

2.4. Water quality analysis

Both the RAW and CTL seawater treatments were sampled weekly for water quality analyses. These analyses included four dissolved inorganic nutrients (DIN): NH₄⁺, PO₄³⁻, NO₂ and NO₃⁻, as well as particulate organic carbon (POC) and nitrogen (PON), dissolved organic carbon (DOC), particulate phosphorus (PPO₄), and the algal pigments; chlorophyll *a* and phaeophyll. Flow cytometry was also conducted to determine the amounts of bacteria-sized cells and virus-sized particles in the CTL and RAW seawater. In the laboratory, the samples were analysed following standard procedures (Furnas and Mitchell, 1996).

2.5. Proximate, lipid class, and fatty acid analysis

All samples were analysed for lipid and ash composition following standardised procedures described previously by Conlan et al. (2014). Briefly, lipids were extracted by cold extraction with dichloromethane:methanol (2,1), while ash was determined by incineration in a muffle furnace (C & L Fetlow, Model WIT, Blackburn, Victoria, Australia) at 450 °C for 12 h. Total protein content was measured using the Kjeldahl method (crude protein calculated as nitrogen × 6.25) in an automated Kjeltech analyser (Tecator, Sweden).

Lipid class analysis was determined using an Iatroscan MK-5 thin layer chromatography-flame ionisation detector (Mitsubishi Chemical Medience, Tokyo Japan) according to the method of Parrish (1999). Briefly, each sample was spotted in duplicate on silica gel S5-Chromarods (5 μm particle size) with lipid separation following a two-step elution sequence: 1) elution of phosphatidylethanolamine (PE), phosphatidylserine and phosphatidylinositol (PS-PI), phosphatidylcholine (PC), and lysophosphatidylcholine (LPC) was achieved in a dichloromethane/methanol/water (50:20:2, by volume) solvent system run to half height (~15 min); and 2) after air drying, elution of wax esters (WAX), triacylglycerol (TAG), free fatty acid (FFA), 1,2-diacylglycerol (1,2DAG), and sterol (ST) was achieved in a hexane/diethyl ether/formic acid (60:15:1.5, by volume) solvent system run to full height (~30 min). During scanning, air and hydrogen flow were kept constant at 2 L min⁻¹ and 155 mL min⁻¹, respectively, while scan speed was 3 mm s⁻¹. The chromatograms were analysed using PowerChrom version 2.6.15 (eDAQ Pty Ltd.). Each lipid class peak was quantified using calibration curves from standards (Sigma-Aldrich Co., USA). Since glycolipids commonly elute with monoacylglycerols and pigments, including chlorophyll, the term “acetone mobile polar lipid” (AMPL) was used in the present study (Parrish et al., 1996). AMPL was quantified using the 1-monopalmitoyl glycerol standard (Sigma-Aldrich Co., USA), which has demonstrated a response that is intermediate between glycolipids and pigments (Parrish et al., 1996). Since AMPL correlated strongly with the phospholipids in the present study (data not shown), this class was grouped in the structural lipid fraction.

Following initial lipid extraction, fatty acids were esterified into methyl esters using an acid-catalysed methylation method and then analysed by gas chromatography as described in Conlan et al., (2014).

2.6. Statistical analysis

Data were analysed statistically using R software version 2.3.1 (R Development Core Team, 2018; RStudio Team, 2018). All available data were considered, and not transformed. Normality and heteroscedasticity were determined by performing the Shapiro-Wilk and Bartlett's tests, respectively. Due to heteroscedasticity, differences between treatments were analysed using a non-parametric Kruskal-Wallis test at a significance level of *P* < .05 (de Menibus, 2015). Where significant differences were detected, a *t*-student *post hoc* test was employed at a significance level of *P* < .05 to determine which groups differed (*agricolae* package (de Menibus, 2015)). Analysis of similarity (ANOSIM) was performed on the FA profiles (% FA) using multiple permutations within a significant fixed effect (*P* < .05) (vegan package

(Oksanen et al., 2017)). The ANOSIM-R value indicates the extent to which the groups differed. Where differences were detected between treatments, similarity of percentages (SIMPER) analyses were conducted to determine which individual classes and FA drove the observed differences (vegan package (Oksanen et al., 2017)). Overall lipid class and FA profiles (% FA) were also visualized with a canonical analysis of principal coordinates (CAP) based on a discriminant analysis (Anderson and Willis, 2003) using a non-parametric Bray–Curtis similarity matrix to visualise the influence of individual FA on the separation of treatments (BiodiversityR package) (Kindt and Coe, 2005). Graphs were prepared using the ggplot2 package (Wickham, 2016).

3. Results

3.1. Water quality analysis

No significant differences were detected in the DIN analyses (Supplementary Table S3). In the particulates, levels of POC, PON, PPO_4^{3-} and the C:N ratio were significantly higher in the RAW water. The algal pigments, chlorophyll *a* and phaeophyll were present in significantly higher concentrations in the RAW, with the CTL concentrations being negligible. The flow cytometry results also showed significantly higher amounts of bacteria-sized cells and virus-sized particles in the RAW treatment ($P < .05$).

3.2. Exogenous diet biochemical analyses

Based on dry weight, the formulated diets (ATF, LONG, and SHORT) contained high levels of total lipid compared to the ALG diet (Table 1). The formulated diets were also higher in protein, being ~five-fold higher than the ALG diet. The ALG and SHORT diets were poorer in storage lipids compared to the ATF and LONG diets, due to high TAG contents in the latter. The ALG and SHORT diets were richer in phospholipids. AMPL was a significant constituent of all diets. All diets contained similar total FA and total SFA concentrations (Table 2). The ALG diet was notably richer in monounsaturated fatty acids (MUFA). On the other hand, the ALG diet contained the lowest PUFA concentration. As formulated, the SHORT diet was deficient in long-chain PUFA (LC-PUFA), containing ~three-fold lower concentrations than the ALG diet, and ~twelve-fold lower concentration than the ATF and LONG diets. Concentrations of the LC-PUFA, 20:5n-3 (EPA), 22:6n-3 (DHA), and 20:4n-6 (ARA) were low or absent in the SHORT diet.

Table 1

Proximate and lipid class composition of exogenous diets fed to captive *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* over 90 days.

Proximate composition (mg g sample ⁻¹ DW)	ALG	ATF	LONG	SHORT
Lipid	81.8 ± 19.3 ^c	212 ± 16.2 ^{bc}	222 ± 1.33 ^a	204 ± 6.03 ^{ab}
Protein	108 ± 8.56 ^b	494 ± 1.74 ^a	490 ± 3.43 ^a	586 ± 6.56 ^a
Ash	305 ± 16.4 ^a	119 ± 18.4 ^b	116 ± 13.0 ^b	78.7 ± 1.73 ^c
Lipid class composition (mg g lipid ⁻¹)				
WAX	36.6 ± 3.57 ^{ab}	29.1 ± 0.05 ^b	27.3 ± 1.69 ^b	45.8 ± 6.82 ^a
TAG	397 ± 28.5 ^c	592 ± 14.3 ^b	644 ± 1.05 ^a	215 ± 10.5 ^d
FFA	129 ± 25 ^a	67.3 ± 5.25 ^{ab}	47.6 ± 4.54 ^b	134 ± 21.3 ^a
1,2-DAG	0 ± 0 ^a	20 ± 14.1 ^a	36.5 ± 0.55 ^a	39.8 ± 28.1 ^a
ST	23.3 ± 5.7 ^{ab}	30.9 ± 1.52 ^{ab}	26 ± 1.26 ^b	37.8 ± 1.34 ^a
AMPL	127 ± 4.96 ^c	152 ± 0.21 ^b	116 ± 1.53 ^d	226 ± 11.5 ^a
PE	85.5 ± 60.4 ^a	56.5 ± 5.71 ^a	27.7 ± 19.6 ^a	80.1 ± 56.6 ^a
PSPI	171 ± 87.8 ^a	0 ± 0 ^a	30.1 ± 21.3 ^a	125 ± 38.5 ^a
PC	31 ± 21.9 ^a	52.2 ± 1.38 ^a	44.6 ± 0.74 ^a	96.6 ± 2.07 ^a
ESTORAGE	562 ± 49.9 ^c	709 ± 5.36 ^b	756 ± 1.25 ^a	434 ± 3.19 ^d
ESTRUCTURAL	438 ± 49.9 ^b	291 ± 5.36 ^c	244 ± 1.25 ^d	566 ± 3.19 ^a
STORAGE:STRUCTURAL	1.35 ± 0.27 ^c	2.44 ± 0.06 ^b	3.09 ± 0.02 ^a	0.77 ± 0.01 ^d

Values are presented as means ± SEM. n = 3.

Table 2

Fatty acid and fatty alcohol composition of exogenous diets fed to captive *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* over 90 days.

Fatty acids (mg g lipid ⁻¹)	ALG	ATF	LONG	SHORT
14:0	72.9 ± 10.2 ^a	33.8 ± 0.25 ^{ab}	34.8 ± 2.18 ^{ab}	3.09 ± 0.09 ^b
16:0	39.2 ± 3.94 ^b	116 ± 0.49 ^a	116 ± 7.33 ^a	89.3 ± 3.17 ^{ab}
18:0	0.98 ± 0.03 ^b	18.3 ± 0.12 ^{ab}	17.5 ± 1.11 ^{ab}	19.2 ± 0.49 ^a
ΣSFA	118 ± 15.2 ^a	177 ± 0.88 ^a	177 ± 11 ^a	130 ± 3.46 ^a
18:1n-9	55.3 ± 8.35 ^a	24 ± 0.23 ^{ab}	20.6 ± 0.25 ^b	47.6 ± 1.52 ^a
16:OH	0 ± 0 ^c	0.28 ± 0.01 ^b	0.36 ± 0.04 ^a	0 ± 0 ^c
ΣMUFA	126 ± 19.4 ^a	46.3 ± 0.52 ^c	38.8 ± 0.42 ^d	61.4 ± 1.54 ^b
18:3n-3	17.8 ± 3.46 ^a	0 ± 0 ^b	0 ± 0 ^b	17.9 ± 0.96 ^a
18:4n-3	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b
20:4n-3	0.56 ± 0.4 ^a	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a
20:5n-3	1.92 ± 0.3 ^b	18.1 ± 0.18 ^a	15 ± 0.34 ^{ab}	1.93 ± 0.04 ^b
22:5n-3	0 ± 0 ^b	0.92 ± 0.01 ^a	0.91 ± 0.05 ^a	0.15 ± 0.1 ^{ab}
22:6n-3	21.3 ± 3.43 ^{ab}	86.9 ± 0.12 ^a	94.4 ± 7.31 ^a	5.23 ± 0.12 ^b
18:2n-6	13 ± 2.4 ^b	8.75 ± 0.08 ^c	7.55 ± 0.22 ^d	155 ± 7.36 ^b
18:3n-6	0.94 ± 0.26 ^a	0.66 ± 0 ^a	0.69 ± 0.05 ^a	10.3 ± 0.26 ^a
20:3n-6	0.2 ± 0.14 ^{ab}	1.09 ± 0.02 ^a	1.2 ± 0.11 ^a	0.16 ± 0.12 ^b
20:4n-6	0 ± 0 ^c	5.65 ± 0.04 ^a	4.98 ± 0.09 ^b	0 ± 0 ^c
22:4n-6	2.24 ± 0.44 ^a	0.21 ± 0 ^a	0.09 ± 0.06 ^b	0 ± 0 ^b
ΣPUFA	68.8 ± 12.4 ^b	155 ± 0.54 ^{ab}	162 ± 10.8 ^{ab}	194 ± 8.17 ^a
TOTAL	315 ± 47.3 ^a	379 ± 1.95 ^a	378 ± 21.4 ^a	385 ± 13.2 ^a
Σn-3 PUFA	45.3 ± 8 ^{ab}	108 ± 0.27 ^a	113 ± 7.27 ^a	26.7 ± 0.74 ^b
Σn-6 PUFA	21.9 ± 4.19 ^b	47.1 ± 0.27 ^{ab}	49.1 ± 3.55 ^{ab}	167 ± 7.43 ^a
Σn-3 LC-PUFA	27.5 ± 4.54 ^{ab}	108 ± 0.27 ^a	113 ± 7.27 ^a	8.82 ± 0.23 ^b
Σn-6 LC-PUFA	6.01 ± 1.12 ^{ab}	37.6 ± 0.11 ^a	40.8 ± 3.34 ^a	0.86 ± 0.1 ^b

Values are presented as means ± SEM. 16:OH = 1-hexadecanol (fatty alcohol). n = 3.

3.3. Growth

No mortality occurred for any species during the experiment. At 90 days, the ALG treatment elicited the highest growth for *A. millepora* and *A. tenuis*, while *A. loripes* recorded similar growth in the ALG and COMB treatments (Fig. 1a–c). For *A. loripes* and *A. tenuis*, growth in the ALG treatment was only significant compared to one other treatment (*A. loripes*: LONG, *A. tenuis*: SHORT). Meanwhile, for *A. millepora*, this was significant compared to the ATF, COMB, LONG, and SHORT treatments, with the lowest growth recorded in the COMB treatment.

3.4. Proximate composition

There were no significant differences in total lipid concentration for

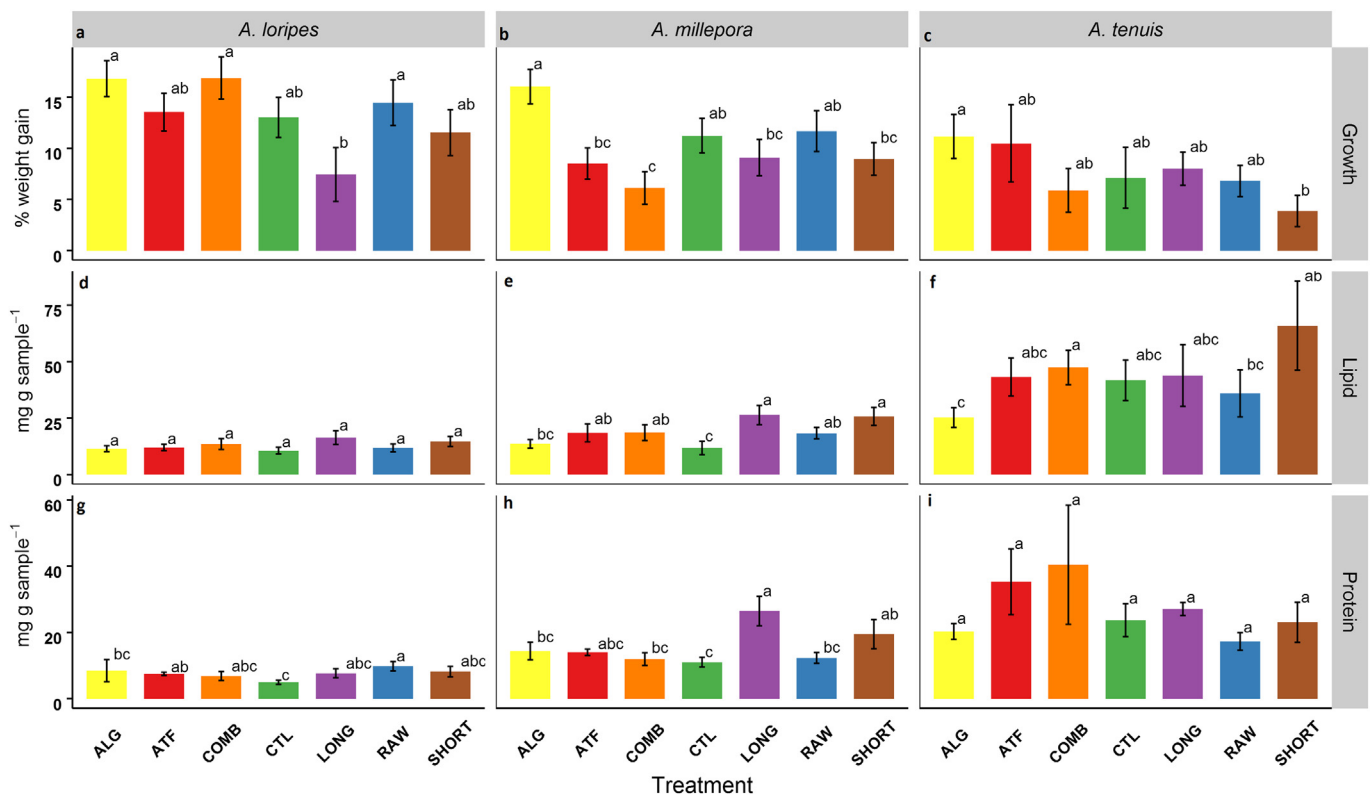


Fig. 1. Growth, and total lipid and protein concentrations of *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* fed seven different feeding regimes over 90 days. Values are presented as means $\pm$ SEM. Values in the same facet that do not share the same superscripts are significantly different ($P < .05$). $n = 15$ nubbins treatment⁻¹ species⁻¹.

A. loripes (Fig. 1d–f). For *A. millepora*, the LONG and SHORT treatments exhibited the highest total lipid concentrations, and these were ~two-fold greater than the lowest amounts observed in the CTL treatment. In *A. tenuis*, the total lipid concentration was highest in the SHORT treatment, and lowest in the ALG treatment.

In *A. loripes*, the total protein concentration was highest in the RAW treatment, and lowest in the CTL treatment (Fig. 1g). The total protein results for *A. millepora* followed the same trend as the total lipid for this species, where the highest concentrations were observed in the LONG treatment and lowest in the CTL treatment (Fig. 1h). There were marked differences in protein for *A. tenuis*, being highest in the COMB treatment, and lowest in the RAW treatment. However, high variability between replicates rendered these differences statistically insignificant ($P > .05$) (Fig. 1i).

Comparing the mean total lipid concentration against the mean weight gain results after 90 days (T2) revealed a negative correlation for all species (*A. loripes*: $r^2 = 0.45$, *A. millepora*: $r^2 = 0.23$, *A. tenuis*: $r^2 = 0.65$), which was significant for *A. tenuis* ($P < .05$) (Fig. 2).

3.5. Lipid class composition

In *A. loripes*, the CTL and ALG treatments contained the highest concentrations of storage lipids, and consequently, the lowest concentrations of structural lipids (Supplementary Fig. S1, and this was significant for all treatments except COMB. The higher concentrations of storage lipids in the CTL and ALG treatments were attributable to significantly higher levels of WAX and TAG. AMPL was the predominant structural lipid class, and this was highest in the ATF, COMB, LONG, RAW, and SHORT treatments.

The similarity of the lipid class profiles (% lipid) of each treatment were determined for each species using one-way ANOSIM (Table 3). For all species, ANOSIM R values showing dissimilarities of > 0.15 were statistically significant. For *A. loripes*, the CTL showed significant

dissimilarities compared to the ATF, LONG, RAW, and SHORT treatments, with the highest being the ATF. Significant dissimilarity was also recorded between the ALG and ATF treatments. In *A. millepora*, the highest dissimilarities were recorded between the RAW treatment and three formulated diets, ATF, LONG, and SHORT. The highest was between the RAW and LONG treatments. Similarly, *A. tenuis* recorded significant dissimilarities between the RAW treatment and all formulated diets, including the COMB treatment. The highest dissimilarity occurred between the RAW and LONG treatments.

Lipid class CAP results were similar for all species, and did not show a distinct separation of individual treatments (Fig. 3a). Thus, all species and treatments were pooled into a single CAP plot. The CAP reflected the ANOSIM results, illustrating the distinct separation of the ‘natural’ treatments (ALG, CTL, RAW) from the ‘formulated’ treatments (ATF, LONG, and SHORT). The SHORT treatment showed the greatest scattering, while the ATF treatment was the most tightly clustered.

The biplot illustrates the relationships between individual lipid class, and their effect on the scoring of each treatment (Fig. 3b). The ‘formulated’ group was largely influenced by PSPI, PE, and AMPL, while the separation of the ‘natural’ group was largely driven by TAG, WAX, PC, FFA, and ST. The percentage contribution (SIMPER) of each class to the overall differences between treatments is represented in the colour gradients of the biplot vectors. Overall, TAG and AMPL were the major contributors to the difference between the ‘formulated’ and ‘natural’ treatments, showing opposing results. This reflects the quantitative concentrations of these classes (Supplementary Fig. S1), since they were generally the most dominant in the lipid class profile.

A. millepora and *A. tenuis* demonstrated a positive correlation between the mean total protein and mean AMPL concentrations (*A. millepora*: $r^2 = 0.91$, *A. tenuis*: $r^2 = 0.51$) (Supplementary Fig. S2), and this was significant for the former ($P < .05$).

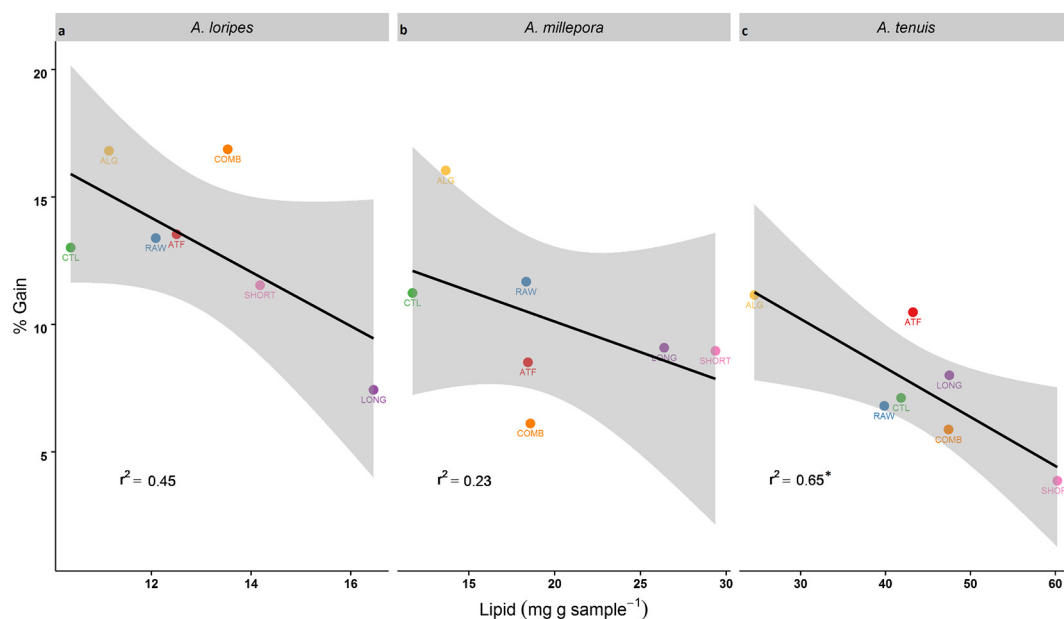


Fig. 2. Correlation between growth and total lipid concentration of *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* fed seven different feeding regimes over 90 days. Values are presented as means. Shading indicates 95% confidence intervals. *Indicates statistical significance ($P < .05$). $n = 15$ nubbins treatment⁻¹ species⁻¹.

Table 3

ANOSIM R values for lipid class profiles (% lipid) in *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* fed seven different feeding regimes over 90 days.

<i>A. loripes</i>	ALG	ATF	COMB	CTL	LONG	RAW
ATF	0.13*					
COMB	0.00	0.05				
CTL	0.00	0.30**	0.11			
LONG	0.03	0.00	0.00	0.16*		
RAW	0.05	0.00	0.00	0.15*	0.00	
SHORT	0.01	0.04	0.00	0.15*	0.00	0.00

<i>A. millepora</i>	ALG	ATF	COMB	CTL	LONG	RAW
ATF	0.09					
COMB	0.00	0.03				
CTL	0.00	0.08	0.00			
LONG	0.16*	0.02	0.16*	0.19**		
RAW	0.00	0.25**	0.04	0.00	0.34**	
SHORT	0.04	0.00	0.06	0.10	0.00	0.27**

<i>A. tenuis</i>	ALG	ATF	COMB	CTL	LONG	RAW
ATF	0.06					
COMB	0.00	0.00				
CTL	0.02	0.00	0.00			
LONG	0.09	0.00	0.00	0.00		
RAW	0.02	0.22**	0.19*	0.20**	0.23*	
SHORT	0.00	0.00	0.00	0.00	0.00	0.09

$P < .001$ '***', $P < .01$ '**', $P < .05$ '*'. $n = 15$ nubbins treatment⁻¹ species⁻¹.

3.6. Fatty acid and fatty alcohol composition

In the ANOSIM results for the fatty acid profiles (% FA), the 'natural' diets (ALG, CTL, RAW) again showed higher similarity with each other, while the 'formulated' diets (ATF, LONG, SHORT) were closely related. For all species, ANOSIM R values showing dissimilarities of > 0.17 were statistically significant (Table 4). For *A. loripes*, the highest dissimilarity values were recorded between the ATF and CTL treatments and the ALG and ATF. In *A. millepora*, only three significant differences were recorded in the ANOSIM results, namely between the ALG and

ATF treatments, the ALG and LONG treatments and LONG and RAW treatments. On the other hand, *A. tenuis* exhibited several significant differences in R values. Of particular note was the ATF treatment, which was significantly dissimilar in comparison to all other treatments and the SHORT treatment, which was significantly dissimilar compared to all other treatments with the exception of the LONG treatment. The highest R value was recorded between the ATF and RAW treatments for *A. tenuis*.

CAP results were similar for all species, and did not show a distinct separation of individual treatments (Fig. 4a). Thus, all species and treatments were pooled into a single CAP plot. The results showed a separation of the 'natural' treatments (ALG, CTL, RAW) from the 'formulated' treatments (ATF, LONG, and SHORT), with the COMB treatment generally lying between. The LONG treatment showed the greatest scattering, while the RAW and SHORT treatments were more closely clustered among themselves.

The biplot shows the top ten individual FA contributing to the overall dispersion of the seven treatments for all species (Fig. 4b). The 'formulated' group was largely influenced by 20:1n-11, 20:5n-3 (EPA), 20:4n-6 (ARA), and 22:4n-6, while the 'natural' group was largely influenced by 16:0, 1-hexadecanol (16:OH), 18:3n-6, and DHA. Overall, 16:0 was the major contributor to the variation between sample points, reflecting its status as the most quantitatively dominant FA, with proportions that were on average an order of magnitude greater than most of the other FA (Supplementary Tables S4–S6). This was followed by EPA, ARA, and 20:1n-11.

4. Discussion

The present study examined seven individual feeding regimes for their effects on the growth and nutritional composition of three captive *Acropora* coral species. Optimising exogenous diets for captive corals has the potential to substantially improve their growth and overall health, ultimately advancing their aquaculture prospects (Grottoli et al., 2006; Leal et al., 2016).

After 90 days, all species demonstrated the highest growth in response to the ALG treatment (*A. loripes* equivalent in COMB treatment), providing clear evidence that phytoplankton can serve as a suitable vehicle for nutrient delivery in *Acropora* corals. Indeed, the weight gain

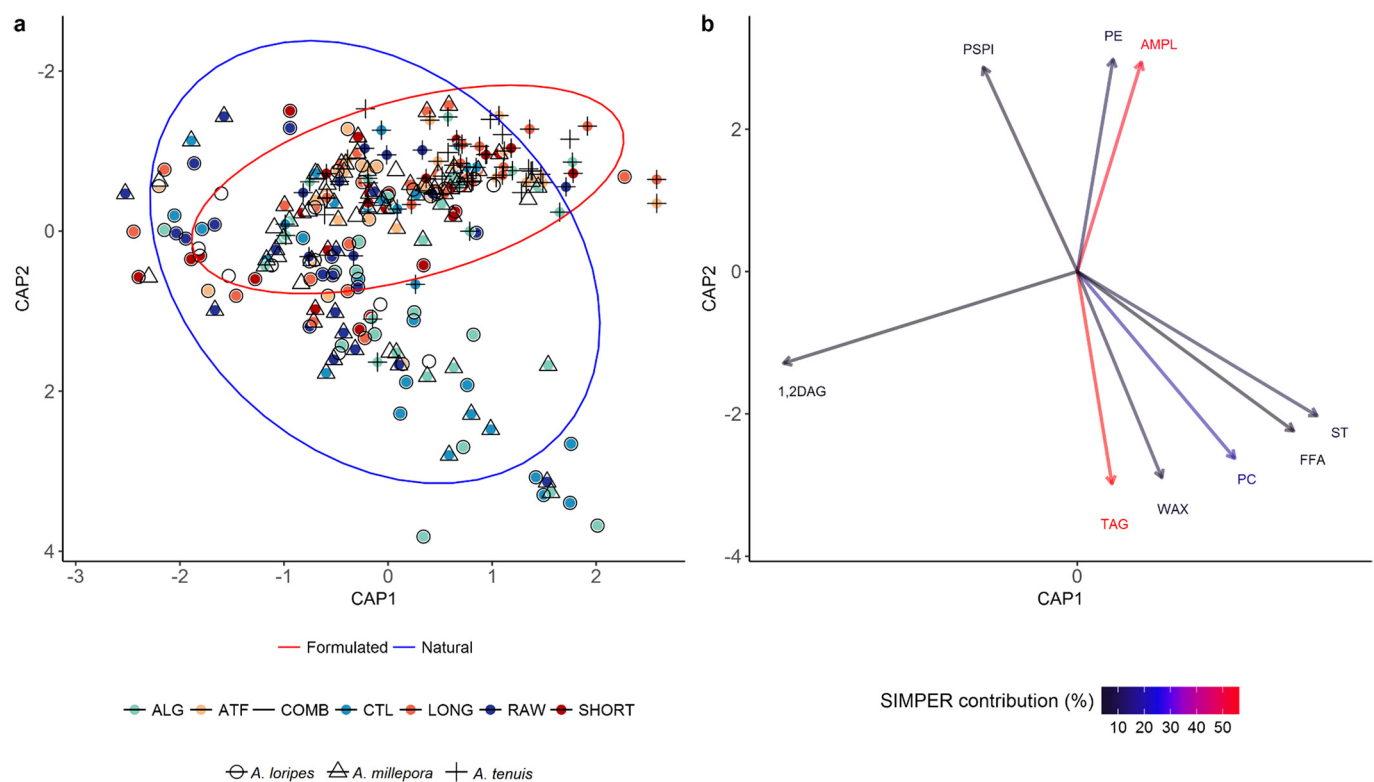


Fig. 3. Canonical analysis of principal components (CAP) a) score plot and b) biplot, showing overall lipid class profile (% lipid) of *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* fed seven different feeding regimes over 90 days. a) Ellipses show 95% confidence intervals of 'formulated' (ATF, LONG, SHORT) and 'natural' (ALG, CTL, RAW) feeding regimes. b) Vectors show individual classes contributing to the overall variance between treatments. Colour gradient shows percentage contribution to overall variance, obtained with SIMPER analysis. $n = 15$ nubbins treatment⁻¹ species⁻¹.

Table 4

ANOSIM R values for fatty acid and fatty alcohol profiles (% fatty acids) in *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* fed seven different feeding regimes over 90 days.

<i>A. loripes</i>	ALG	ATF	COMB	CTL	LONG	RAW
ATF	0.37**					
COMB	0.06^^	0.18***				
CTL	0.05^^	0.37***	0.04			
LONG	0.23**	0.04***	0.05	0.26**		
RAW	0.18**	0.02***	0.00	0.18**	0.04	
SHORT	0.19**	0.00***	0.05	0.19**	0.06	0.02

<i>A. millepora</i>	ALG	ATF	COMB	CTL	LONG	RAW
ATF	0.18*					
COMB	0.02^	0.05				
CTL	0.06^	0.07	0.07			
LONG	0.18*	0.04	0.09	0.04		
RAW	0.02^	0.12	0.05	0.01	0.18*	
SHORT	0.16^	0.05	0.01	0.06	0.00^	0.14

<i>A. tenuis</i>	ALG	ATF	COMB	CTL	LONG	RAW
ATF	0.36**					
COMB	0.12^^	0.26***				
CTL	0.08^^	0.42***	0.07^			
LONG	0.08^^	0.24***	0.01^	0.06^		
RAW	0.10^^	0.53***	0.08^	0.06^	0.11	
SHORT	0.19^^	0.19***	0.19*	0.20*	0.05	0.40**

P < .001 '***', P < .01 '**', P < .05 '*'. $n = 15$ nubbins treatment⁻¹ species⁻¹.

for *A. millepora* in the ALG treatment ($16 \pm 2.85\%$ gain) was almost double that achieved with *Artemia* nauplii in the same species over an equivalent experimental period ($8.95 \pm 3.76\%$ gain) (Conlan et al., 2018). This lends support to the aforementioned hypothesis that *Acropora* corals prefer smaller, less motile prey. Moreover, growth in the ALG treatment was greater than for the RAW treatment in the present study suggesting a) the nutritional composition of the ALG diet was superior to the naturally available food in the RAW treatment, b) the algal density in the ALG treatment surpassed that in the RAW - allowing greater consumption, or c) the algal species composition in the RAW treatment may not have been as digestible as the ALG treatment (*I. galbana* is readily digestible due to its small size and lack of cell wall (Wikfors and Patterson, 1994)).

The growth of *A. millepora* in the ALG treatment was significantly higher than the ATF, COMB, LONG, and SHORT treatments. This again indicates a low tolerance or acceptance of formulated diets in *A. millepora*, which agrees with the findings of Conlan et al., (2018). Poor growth and development has also been recorded in captive sea urchin larvae (*Paracentrotus lividus*) fed formulated diets compared to microalgae (Liu et al., 2007). This may be due to reduced feeding stimulants in the formulated diets compared to the live algae, such as palatability, enzymatic activity and chemical attractants (Cahu and Zambonino Infante, 2001). Furthermore, notable differences were recorded between the bound LC-PUFA-rich treatment (ATF) when compared to the unbound version (LONG) for *A. millepora*. In particular, the total lipid concentration of *A. millepora* in the LONG treatment was ~43% higher than the ATF treatment, and ~88% higher for total protein. Although not statistically significant, this suggests that *A. millepora* may prefer to receive dietary nutrients in dissolved form or as small, unbound particles. Alternatively, this may indicate reduced digestibility of the ATF diet due to the sodium-alginate binder, as has been shown in larval fish (Cahu and Zambonino Infante, 2001). However, the lipid class and fatty

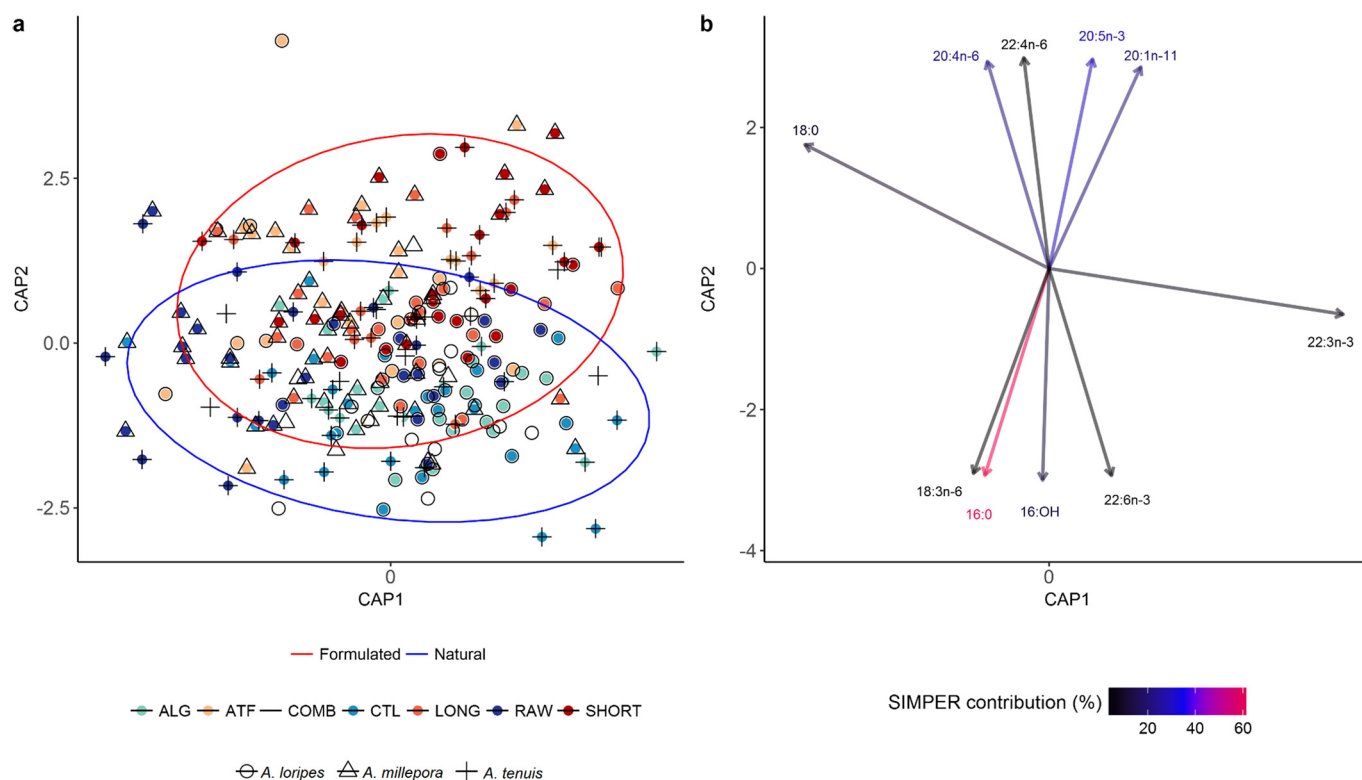


Fig. 4. Canonical analysis of principal components (CAP) a) score plot and b) biplot, showing overall fatty acid and fatty alcohol profile (% fatty acids) of *Acropora loripes*, *Acropora millepora*, and *Acropora tenuis* fed seven different feeding regimes over 90 days. a) Ellipses show 95% confidence intervals of 'formulated' (ATF, LONG, SHORT) and 'natural' (ALG, CTL, RAW) feeding regimes. b) Vectors show top ten individual fatty acids and fatty alcohols contributing to the overall variance between treatments. Colour gradient shows percentage contribution to overall variance, obtained with SIMPER analysis. $n = 15$ nubbins treatment⁻¹ species⁻¹.

acid compositions showed few significant differences between the ATF and LONG diets, indicating inhibited nutrient absorption in the ATF treatment on a quantitative basis only.

The growth results in the ATF treatment for *A. millepora* ($8.51 \pm 2.16\%$ gain) were also markedly higher in the present study compared to that recorded by Conlan et al., (2018) with the same quantity of ATF diet delivered as twice daily meals rather than continuously ($3.72 \pm 1.99\%$ gain). This corresponds to previous findings that scleractinian corals are generally opportunistic feeders, whose exogenous feed intake is largely dependent upon availability (Nahon et al., 2013). Indeed, Liu et al. (2007) found that captive sea urchin larvae performed better on formulated diets when the feeding rate was increased from twice to three times daily. Moreover, compared with meal feeding, continuous feeding allows a slow, even influx of nutrients to the digestive system, improving digestion and assimilation efficiency (Cui et al., 1997), which may be advantageous given corals' rudimentary digestive capabilities (Agostini et al., 2012).

Interestingly, many of the results obtained for *A. millepora* in the present study differed from the other species examined. For example, in contrast to *A. millepora*, the growth of *A. loripes* and *A. tenuis* in the ALG treatment was only statistically significant compared to one other treatment (*A. loripes*: LONG, *A. tenuis*: SHORT). Moreover, *A. loripes* recorded negligible differences in total protein and lipid concentrations between the ATF (bound) and LONG (unbound) treatments, and *A. tenuis* had higher protein in the ATF treatment compared to the LONG. This may indicate greater digestive capabilities in these species compared to *A. millepora*, a preference for bound particulates, or greater suitability of the formulated diets' nutritional profiles for these species. Regardless, these results demonstrate a marked difference in the dietary requirements of coral species within a single genus. This has implications for aquaculture, since dietary formulations may require specific tailoring to meet the requirements of individual species.

Notably, all species exhibited a negative correlation between total lipid concentration and weight gain, providing clear evidence that high dietary lipid levels are inappropriate for *Acropora* corals. Informatively, the treatments that generally elicited the highest lipid content and subsequent lowest growth were the 'formulated' diets, namely ATF, LONG, and SHORT (also COMB for *A. millepora* and *A. tenuis*), which contained more than two-fold the total lipid content of the ALG diet. A growth depressing effect of high dietary lipid levels have previously been recorded in marine fish larvae (Izquierdo et al., 2000; Morais et al., 2007). This is particularly pronounced with high dietary levels of storage lipids (WAX, TAG, FFA, and 1,2DAG), corresponding to the formulated diets in the present study, which contained ~43–76% storage lipids.

There are several possible explanations for why elevated concentrations of dietary lipid may depress coral growth. Firstly, high lipid intake may cause reductions in digestive efficacy, digestive enzyme activity, and absorption efficiency (Morais et al., 2007). Secondly, high lipid may cause lower food consumption due to the need to regulate dietary energy intake (Morais et al., 2007). This would also reduce the intake of dietary protein required for new tissue synthesis and thus depress growth. Thirdly, high dietary lipid may cause an accumulation of large lipid droplets in absorptive cells, which may in turn reduce FA absorption efficiency and cause reduced synthesis rates of new cellular lipids for growth, as observed for other organisms (Morais et al., 2007).

In addition, it has previously been shown that at excessive dietary protein levels, protein is no longer used for increasing weight gain in young fish, but its individual amino acids might be deaminated and their carbon components used as energy, leading to fat deposition (Meyer and Fracalossi, 2004). This is again applicable to the formulated diets in the present study, since they were comparatively rich in protein (~490–586 mg g sample⁻¹). On the other hand, enzyme activity is stimulated by low protein diets such as phytoplankton, optimising

protein assimilation (Jones et al., 1997). Correspondingly, although the total protein content of the ALG diet was ~one fifth that of the formulated diets, the corals subjected to this treatment exhibited protein concentrations that were similar to most other treatments. Importantly, the protein levels of corals in the ALG treatment were coupled with high growth. Indeed, high protein levels generally signify actively growing sites in corals, where protein synthesis and retention facilitates calcification, tissue synthesis, and increased mitotic rates (Hemond et al., 2014; Ramos-Silva et al., 2014). On the other hand, high protein coupled with comparatively low growth, such as exhibited by the LONG treatment for *A. millepora* and the COMB and SHORT treatments for *A. tenuis*, indicates stress and nutritional imbalance (Unnikrishnan and Paulraj, 2010), since catabolism of excess protein can generate toxic levels of ammonia and free amino acids, which may impact normal metabolism and growth (Unnikrishnan and Paulraj, 2010; Yang et al., 2002).

Surprisingly, despite the ample provision of storage lipids, particularly TAG, in the ATF and LONG diets, the coral in these treatments were dominated by AMPL, constituting almost half the total lipid concentration and subsequently boosting the structural lipid portion. Structural lipids such as ST and phospholipids are major constituents of cell membranes and intracellular organelles (Lall, 2002). Meanwhile, storage lipids such as WAX and TAG provide readily catabolised energy compounds (Lin et al., 2013). Therefore, during stress, increased structural relative to storage lipids generally reflects catabolism of high-energy classes such as WAX and TAG for energy, resulting in a larger proportion of indispensable, membrane-bound structural lipids and reduced total lipid concentration (Lin et al., 2013). However, in the present study, the treatments recording the highest amounts of structural lipids also exhibited the highest total lipid concentrations, indicating lipid accumulation in the form of AMPL.

Interestingly, AMPL accumulation has been linked to dietary N levels in marine invertebrates. For example, Gordillo et al. (2001) showed that in green macroalgae, the AMPL fraction was clearly predominant when N was not limiting, while Parrish (1999) found that juvenile scallops fed N-limited algae showed a large decrease in AMPL. Further, Parrish (1999) found the greatest decreases in AMPL concentrations occurred in the scallops recording the greatest growth. This corresponds to the findings of the present study, whereby AMPL concentrations showed positive correlations with total protein (i.e. nitrogen) for *A. millepora* and *A. tenuis*, and were generally lowest in the treatments exhibiting the greatest growth for all species. This may again indicate deamination of excess dietary protein, causing increased amino acid catabolism and conversion to lipid (Refstie et al., 2004) in the form of AMPL. However, further investigation is required.

The lipid class CAP showed that the treatments that generally elicited a positive response for most species (ALG, CTL, RAW) were influenced by storage lipids, largely in the form of WAX and TAG. This represents larger energy reserves in these treatments, which also showed greater similarity to documented storage: structural lipid ratios in wild *Acropora* corals (Hinrichs et al., 2013). Since TAG possesses three esterified FA, while ST, phospholipids, and the constituents of AMPL have two or less (Lee et al., 2006), the treatments exhibiting the highest TAG concentrations for each species also recorded the highest total FA concentrations. Moreover, SFA and MUFA are mainly stored as WAX and TAG in corals (Rodrigues et al., 2008), and these groups were correspondingly found in the highest concentrations in the same treatments. SFA and MUFA also represent readily catabolised energy sources (Tocher, 2003), again demonstrating the greater abundance of high-energy reserves in the natural treatments.

The FA CAP analyses showed that the individual FA, 16:0, 18:3n-6, and DHA, as well as the fatty alcohol, 16:OH, were strongly correlated with the 'natural' treatments across all species examined. This is again consistent with the higher abundances of WAX and TAG, since 16:0 and 18:3n-6 are generally moieties of these classes in cnidarians (Imbs et al., 2015; Yamashiro et al., 1999), and fatty alcohols are generally

esterified to WAX (Tolosa et al., 2011). Meanwhile, the abundance of DHA in the best performing treatments for each species correlates to the findings of Conlan et al. (2018), whereby *A. millepora* exhibited significantly higher levels of DHA in the RAW treatment. However, despite the apparent health-promoting effects of DHA in *Acropora* corals, the abundant provision of this FA in the ATF and LONG diets did not result in superior performance, or even increased DHA deposition. This mirrors the results of the ATF treatment in Conlan et al. (2018), and supports the aforementioned theory that this FA is largely obtained through *de novo* synthesis from endosymbionts of the Symbiodiniaceae family, the host, or other endogenous algae and bacteria (Imbs et al., 2010, 2009; Monroig et al., 2013). As such, the FA composition of the natural treatments may reflect increased symbiont densities (Imbs et al., 2007), which agrees with the findings of Conlan et al. (2018) in the RAW treatment. This is further strengthened by the large influence of 18:3n-6 on the natural treatments, since this FA has been shown to be abundant in *in hospite* symbionts (Chen et al., 2015). Moreover, the n-6 PUFA, ARA and 22:4n-6, which were major contributors to the separation of the formulated treatments from the natural, have been shown as chemotaxonomic biomarkers for the coral host (Chen et al., 2015; Conlan et al., 2017b; Revel et al., 2016), suggesting a predominance of animal tissues in these treatments.

Notably, several of the FA contributing to the separation of the formulated treatments from the natural, including EPA and ARA, are generally esterified to phospholipids (Estévez et al., 1999), correlating to the higher abundance of phospholipids in these treatments. Since EPA and ARA are known eicosanoid precursors, which are critical for numerous physiological processes, including pigmentation and immune function (Sargent et al., 1999), their strong influence over the formulated treatments may reflect selective retention to preserve the most essential components of biological membranes during nutritional stress (Izquierdo et al., 1996).

This is supported by the results of the SHORT treatment. Indeed, despite the SHORT diet containing no detectable levels of ARA, and comparatively low levels of EPA and DHA, these were present in the SHORT treatment for all species, and recorded few significant quantitative differences compared to the other treatments. Although *de novo* synthesis of these FA in the SHORT treatment cannot be ruled out at this stage, it is refuted by the strong influence of host-associated FA on the SHORT treatment, which may indicate reduced symbiont densities. Furthermore, there is little evidence of quantitative increases in intermediate FA on the n-3 and n-6 biosynthetic pathways. Interestingly, although the performance of the SHORT treatment was comparatively poor for all species, it must be noted that no mortalities occurred in this treatment, and all species recorded weight gain after 90 days. As such, the results of the present study indicate that dietary inclusion levels of total protein and lipid concentrations are greater determinants of success in coral diets than individual FA.

Clearly, considerable work is still required to optimise formulated diet compositions for captive *Acropora* sp. to achieve growth and health levels akin to those possible with comparatively natural feeding regimes. Future studies are encouraged to expand upon this work, particularly concerning optimal dietary levels of macronutrients. On the other hand, the present study indicates that phytoplankton can serve as a suitable vehicle of nutrient administration for captive *Acropora* corals. Further investigation in this area, possibly pertaining to varying algal densities and species, would greatly advance the elucidation of an optimised feeding regime for captive coral rearing.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

This project was conducted at The Australian Institute of Marine Science (AIMS) and Deakin University. The authors wish to thank the SeaSim team at AIMS and the staff of Deakin University's School of Life and Environmental Sciences for technical assistance throughout the project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2019.734420>.

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