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REPORT

Lipid composition of coral propagules and reproductive material in coral restoration nurseries

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Abstract Coral restoration efforts have rapidly increased worldwide, including the development of several programmes on the Great Barrier Reef (GBR) in recent years. While many restoration programmes utilise in-water nurseries to accelerate coral biomass yields, the impact of nursery environments on propagule quality has not been examined despite the importance of coral fitness for ensuring resistant populations. Here, we investigated two fitness indicators (lipid diversity and tissue protein abundance) of *Acropora millepora* adults and eggs grown on coral nurseries versus native reef on the GBR, with adults assessed at two sites (Blue Lagoon and Rayban) and eggs assessed at one site (Blue Lagoon). Lipid profiles of adult colonies varied by site and origin (nursery versus wild reef), with adult nursery corals exhibiting an elevated relative abundance of storage lipids (diacylglycerols and triacylglycerols) and lipid classes responsible for regulating membrane structure (phosphatidylcholines and sterol esters), while wild corals were characterised by a greater relative abundance of fatty acids and classes involved in immunoregulation. Comparing eggs from different origins, nursery offspring were richer in energy-storing triacylglycerols, as well as ceramides and phosphatidylcholines essential for membrane structure, while wild eggs had a greater relative abundance of wax ester species also important for energy storage. No differences were found in total protein abundance (adult or eggs) or egg physical characteristics (count and size) between nursery and wild origins. Variations in lipid profiles are consistent with differences in environmental conditions between reef sites and origin (nursery versus wild), highlighting the need to consider site selection and propagation conditions when planning restoration projects. Importantly, these findings demonstrate that the lipid classes with the highest relative abundance in *A. millepora* nursery and wild eggs differed from those in adults from the same origin, suggesting that propagation origin is more important for driving lipid profiles in coral eggs compared to parental effects.

Keywords Lipidomics · Coral nursery · Coral fitness · Reef restoration · Acropora millepora

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Introduction

Coral restoration efforts worldwide have rapidly accelerated over the last decade as a means to augment coral cover, increase species diversity or support ecosystem function on reefs suffering local or anthropogenic impacts (Boström-Einarsson et al. 2020; Lirman and Schopmeyer 2016; Ware et al. 2020). Various techniques have been trialled for coral restoration (e.g. larval seeding, selective breeding) and remain in different stages of feasibility (Suggett and van Oppen 2022). However, propagation of asexual coral fragments in a nursery setting (*in situ* or *ex situ*) followed by

transplantation back onto the reef—the process commonly referred to as "coral gardening"—remains the most common practice in place to increase coral biomass (Boström-Einarsson et al. 2020; Rinkevich 1995; Shaish et al. 2008; Suggett and van Oppen 2022). Many studies have demonstrated that corals reared on nurseries have enhanced growth and sexual reproduction (i.e. fecundity and larval release) compared to parallel colonies growing on the reef (Horoszowski-Fridman et al. 2020; Ishida-Castañeda et al. 2020; Nuñez Lendo et al. in press; O'Donnell et al. 2017; Schopmeyer et al. 2017), likely due to a variety of factors including increased water-flow, greater resource delivery and reduced predation (Afiq-Rosli et al. 2017; Shafir et al. 2006b), resulting in improved resource acquisition and in turn faster growth. As such, coral nurseries offer a valuable tool to support rapid production of coral biomass without impacting the natural system through removal of native colonies.

Coral nurseries are most frequently used for the growth of asexual coral propagules (Boström-Einarsson et al. 2020; Edwards and Gomez 2007); however, the accelerated growth of corals raised on nurseries also provides an opportunity for colonies to reach reproductive size more rapidly, where sexual maturity is linked to size in many modular organisms, including some coral species (Amar and Rinkevich 2007; Guest et al. 2014; Kai and Sakai 2008; but see Rapuano et al. 2023). Coral nurseries therefore also provide a time and cost-effective approach to augment sexual reproduction through accelerated maturation, but whether this comes at a cost to other life-history traits such as propagule or egg energetics remains unknown. Both asexually and sexually generated propagules (herein also known as coral material) can be returned to the reef through restoration workflows, such as outplanting (Boström-Einarsson et al. 2020; Suggett et al. 2019) or larval "seeding" (Heyward et al. 2002; Omori 2005), but also naturally via fragmentation or sexual reproduction of mature outplanted colonies (Chamberland et al. 2016; Guest et al. 2023). As such, it is important to understand reproductive and physiological traits of coral material propagated for restoration to validate how this biomass can contribute to reef resistance, recovery and function of any given coral population. Such a critical notion requires understanding the efficacy of coral nurseries based on biological metrics beyond growth and survivorship of adult colonies (e.g. Nuñez Lendo et al. in press), which to date are the most commonly used parameters to evaluate the "success" of restoration programmes (Hein et al. 2017).

Of corals' many biochemical constituents, lipids are a key health indicator for both eggs and mature colonies (Grottoli et al. 2004; Padilla-Gamino et al. 2013; Rivest and Hofmann 2015). Extent of tissue energy reserves is crucial for the resistance and resilience of the coral host during periods of heightened stress (e.g. bleaching events; Tagliafico et al. 2017; Towle et al. 2015), and the catabolism of lipids required to maintain fitness can result in colony mortality if reserves are depleted (Anthony et al. 2009; Grottoli et al. 2004; Schoepf et al. 2015). Despite this, adult colonies have been found to prioritise allocation of autotrophically acquired carbon to gamete development rather than tissue maintenance under stressful conditions (Rodrigues et al. 2022). Lipids are also vital to coral offspring survival, especially lecithotrophic species, providing essential energy during the planktonic phase where larvae benefit from on endogenous reserves to support long-term dispersal and costly developmental phases (Arai et al. 1993; Figueiredo et al. 2012; Harii et al. 2007). Coral eggs and larvae can obtain necessary carbon and nitrogen from their parent colony (stored primarily as lipids and proteins), or through the presence of Symbiodiniaceae following release (Kopp et al 2016), with recent work revealing translocated carbon from parent colonies was almost exclusively autotrophically acquired, while nitrogen was only heterotrophically acquired (Jaffe et al. 2023; Rodrigues et al. 2022). Additionally, lipid classes are known to have distinct functional roles (Bergé and Barnathan 2005), and thus, variations in lipid composition can provide insight on the developmental and functional strategies of different coral species at various life stages (Imbs et al. 2021), as well as metabolic changes driven by distinct environments (Rodrigues et al. 2008). For example, energy-storing lipids such as tri/diacylglycerols and wax esters play distinct roles in larval dispersal and development, whereby triacylglycerols are important for short-term development (Harii et al. 2007), and wax esters constitute a long-term energy source necessary for sustaining dispersal (Arai et al. 1993; Lee et al. 2006). Polar lipids such as ceramides and phospholipids are essential components of membrane structure and can influence the ability of corals to tolerate temperature extremes through fluidity of the lipid membrane (Botana et al. 2022; Lin et al. 2012). Thus, characterising the lipid composition of nursery-grown corals provides one means of assessing the effects of propagation practices on the energetic fitness coral material, an important consideration as coral restoration projects become increasingly prevalent worldwide.

In addition to biochemical indicators, physical characteristics of coral eggs and larvae can provide useful insights on life-history strategies or trade-offs in resource allocation by parent colonies. For example, larger egg size can indicate greater resource investment by parent colonies as a means to fuel dispersal and enhance post-settlement survival, thereby increasing the likelihood of reproductive success (Harii et al. 2002; Hartmann et al. 2013; Isomura and Nishihira 2001; Jones and Berkelmans 2011). Furthermore, reductions in egg size and volume have been observed in response to unfavourable or stressful environmental conditions (e.g. sedimentation, elevated nutrients, or thermal events) impacting parent fitness prior to or during gametogenesis (Cox and

Ward 2002; Hartmann et al. 2018; Hazraty-Kari et al. 2022; Leinbach et al. 2021). As such, variations in physical characteristics such as egg size or volume could also provide a proxy for reproductive success and information on whether key life-history traits are impacted by differences in environmental conditions, such as those potentially experienced on coral nurseries.

While coral nurseries are a useful approach for fast-tracking coral growth and boosting local population recovery through increased availability of outplant material (Peterson et al. 2023), sexual propagation remains crucial for maintaining genetic diversity and system resilience (Baums et al. 2022). However, trade-offs between nursery growth and potential impacts on the energetic quality and physical characteristics of sexually produced offspring have not been examined on the Great Barrier Reef despite important implications for population resilience. Thus, to assess potential impacts of nursery propagation on coral propagules, the aims of this study were to determine: 1) how energetic qualities (lipid composition and protein concentration) varied in adult corals reared on nurseries versus the reef (hereafter termed wild colonies) through characterisation at two distinct reef sites, 2) whether these patterns in energetic qualities were reflected in the eggs of nursery versus wild adult colonies at one representative site and 3) whether increased physical eggs traits (size and number of eggs per bundle) were observed alongside elevated energetic attributes to ultimately assess potential impacts of nurseries on coral propagule fitness. By characterising the lipidome of both asexual and sexual coral propagules reared within a nursery setting, this study aimed to expand our understanding of coral biochemistry and ecology necessary to better inform restoration practices.

Methods

Site selection and collection of material

In November 2021 at Opal Reef (16°13′S 145°53.5′E, an offshore reef 55 km north-east of Port Douglas, northern GBR), fragments of adult *Acropora millepora* corals were collected from nursery-reared and wild colonies (growing at the same depth, 4 m ± 1 m) to assess tissue energetics (lipid composition and protein content). The broadcast spawning coral *A. millepora* was selected as it is a well-studied, highly common species known to grow in a range of reef habitats and depths along the length of the GBR, has a relatively well-known spawning period and is often used in restoration efforts due to its ubiquitous nature (Roper et al. 2022; Tan et al. 2016; van Oppen et al. 2011). Fragments were collected from both propagation sources (nursery and wild colonies) at two reef sites (Blue Lagoon $n = 6$ nursery, $n = 5$ wild; Rayban $n = 8$ nursery, $n = 8$ wild) to assess if differences in energetic quality varied by origin and site. From here on, "origin" will be used to describe the propagation source (i.e. asexual (adults) or sexual (eggs) propagules grown on coral nurseries vs wild reef), while "site" will be used to describe the two natural, distinct reef locations (detailed below) from which adult fragments were collected on Opal Reef (i.e. Blue Lagoon vs Rayban; Fig. S1). Fragments were collected from non-gravid *A. millepora* colonies (visually inspected for gametes with none observed as spawning had occurred the night previous) that had been reared on nurseries for 24–36 months (20–30 cm in diameter) alongside wild colonies of similar diameter growing on the reef (within 50 m of the nurseries) at both sites (Blue Lagoon and Rayban). Fragments 4–5 cm in length were collected from colonies using a hammer and chisel and placed directly in sterile plastic bags. Once above water, fragments were wrapped in aluminium foil and immediately frozen in liquid nitrogen. Fragments were stored at −80 ºC until further processing. To characterise temperature regimes at both sites, temperature loggers (OneTemp HOBO pendant temperature data logger, accuracy ± 0.53 ºC) measuring every hour were deployed at Blue Lagoon and Rayban for a 10-month period (November 2021–August 2022). Furthermore, environmental parameters collected by Strudwick et al (2023) at the same sites over a 14-d period were utilised to provide additional information on light attenuation and water flow. Blue Lagoon (16°12′19.8″S, 145°53′56.5″E), located on the northern end of Opal Reef, is adjacent to a deep channel (Fig. S1) and is characterised by higher water flow, greater light attenuation (decreased water clarity) and lower mean water temperature (average temperature 29.80 ºC, temperature range 6.89 ºC) compared to Rayban (Table S1.A; Strudwick et al. 2023). Conversely, Rayban (16°13′24.6″S, 145°53′24.7″E) is characterised as a coral garden site located near the middle of Opal Reef (Table S1.A), which has lower mean water flow and light attenuation (Strudwick et al. 2023), and greater mean temperature (30.36 ºC) and temperature range (9.16 ºC; Fig. S1).

To determine energetic quality (lipid composition and protein content) and physical characteristics (egg count and size) of sexually produced coral offspring, gravid adult colonies of *A. millepora* were collected during the November 2021 mass spawning. Adult colonies of *A. millepora* growing on nurseries for 24–36 months (20–30 cm in diameter) were collected along with wild colonies of similar size growing on the reef (within 50 m of the nurseries; $n = 3$ per treatment) at Blue Lagoon as gravid colonies were only observed at Blue Lagoon at the time of sampling. Colonies were placed in separate 70 L holding tanks (one for nursery and wild colonies, respectively) containing seawater at 19:00 local time in preparation for spawning. *A. millepora* nursery and wild collected colonies started releasing egg-sperm

bundles at approximately 21:30 local time, five nights after the November 2021 full moon. Colonies were spaced apart within the holding tanks, and egg-sperm bundles were visually tracked as they were released by each colony and collected from the surface of the tanks using Pasteur pipettes to ensure an equal proportion were collected from each colony. Eggs were placed on fine mesh sieves and rinsed with filtered seawater (0.2 µm) to break apart bundles and remove sperm. Once drained of all seawater, eggs were collected in 2 mL cryovials that were immediately wrapped in aluminium foil and frozen in liquid nitrogen for future lipid and protein analysis. To assess physical traits, eggs from the same tanks were pipetted into individual wells within a 6-well plate and gently agitated using a pipette to break apart bundles. The number of eggs contained within each bundle were counted using an Olympus stereo microscope (model SZ61) on 4 × magnification ($n = 10$ per treatment) on the evening of spawning. Following counting, each egg was photographed on the same magnification to allow eggs size to be compared between treatments ($n = 23$ nursery, $n = 20$ wild). Egg size was subsequently quantified using ImageJ (version 1.53) by measuring the longest diameter scaled using a ruler. Temperature loggers (OneTemp HOBO pendant temperature data logger, accuracy ± 0.53ºC) measuring every hour were also installed on nursery frames and the reef (within 50 m of collected material) for 3 d during the mass spawning event to help characterise temperature profiles of source origins at Blue Lagoon (Fig. S1.B).

Adult coral and egg processing

Adult coral fragment tissue (Blue Lagoon $n = 11$, Rayban $n = 16$) was stripped while on ice using an airbrush and Milli-Q water following Johannes and Wiebe (1970). The resulting homogenate was separated into coral host and symbiont components by duplicate rounds of centrifuging at 3000 × g for 5 min at 4 ºC as per Matthews et al. (2023). Total protein content within the host tissue was then quantified using Bradford assay (Bradford 1976). After digesting tissue with 0.2 M NaOH at 98 ºC for 20 min, triplicate 10 µL aliquots of each sample (diluted to 50%) were pipetted into a 96-well plate alongside six serial dilutions of Sigma bovine serum albumin of known concentrations: 1, 0.5, 0.25, 0.125, 0.06, 0.031 and 0 mg ml^{-1}. 250 µL of Bradford reagent was added to each well resulting in a colorimetric assay of protein concentration within the samples. The 96-well plate was gently agitated on a rotating platform for 10 min prior to being analysed on a spectrophotometer at 595 nm (Tecan Spark Microplate Reader). Protein concentration of each sample was calculated from the equation of the protein standard curve and normalised by surface area of the coral skeleton (retained following tissue striping), as determined via the wax dipping method modified from Stimson and Kinzie (1991). Following tissue stripping, paraffin wax was melted in a beaker placed in a hot water bath maintained at 60 ºC to ensure consistency in the viscosity of the wax. Using tweezers, each coral skeleton was dipped into the wax for approximately 2 s and any excess wax removed by gentle shaking. Following a cooling period of approximately 2 min, the skeleton was weighed and dipped a second time using the same procedure. The change in weight between the first and second wax dip was used to calculate the surface area of the coral sample via the equation of a standard curve created from seven calibration objects with known surface areas and weights. Egg protein content was quantified from 100 µL of thawed eggs using the same assay as described above, diluted to 10% of their original concentration.

Lipids were extracted from the coral host tissue and eggs using a modified protocol originally described by Matyash et al. (2008). The exact volume of coral tissue containing 50 µg of protein was collected from each adult sample (as identified via the protein assay) and resuspended in Milli-Q up to 200 µL. Methanol (30 µL) containing 5 µL internal standards for injection normalisation (Avanti EquiSPLASH) was added to the sample, vortexed for 1 min and incubated on ice for 10 min, with two more rounds of vortexing at 3 and 6 min. 1 mL of methyl tert-butyl ether (MTBE) was added, and samples were placed on a shaking platform at 4 ºC for 1 h. To induce phase separation, 250 µL of liquid chromatography (LC) grade chilled water (4 ºC) was added and samples incubated on ice for 10 min before centrifuging at 1000 g for 10 min at 4 ºC. The top layer (1 mL) containing the lipid phase was then carefully removed and dried under a nitrogen steam for approximately 45 min. Dried lipids were stored at − 80 ºC until analysis, and the bottom layer and symbiodiniaceae pellet were also retained and stored at -80 ºC. The coral egg lipid extraction followed the same protocol, except 100 µL of coral eggs (dried on filter paper to remove any seawater) was homogenised with the methanol standard mix using a Chattaway spatula. Following the addition of methanol (plus internal standard), the protocol remained the same. Briefly, the remaining extracts were removed, 1 mL 0.2 M NaOH added to the tissue pellet, and samples vortexed and incubated at 98˚C for 20 min. Samples were centrifuged at 3000 g for 10 min, and 10 µL of the supernatant used for Bradford assay, as above.

Lipid analysis

Prior to analysis, samples were resuspended in 100 µL 2:1 isopropanol methanol (IPA/MeOH) and transferred to autosampler glass vials with a 125 µL glass insert. A pooled sample was created to assist with compound analysis, by combining 5 µL of each sample into a single tube and 100 µL of this pooled sample transferred to an autosampler vial with a 125 µL glass insert. 5 µL injections of

each sample were processed via Liquid Chromatography Mass-Spectrometry (Thermo Orbitrap LC–MS) in positive and negative ion mode. LC–MS was chosen as it allows for analysis of a broad range of lipids (i.e. volatile, non-volatile and thermally sensitive lipids) and is capable of comprehensive lipid profiling and identification using publicly available libraries (Dasilva et al. 2019). Each sample was run as per methodology developed by Violi (2022), in positive mode under acidic chromatographic conditions, and in negative mode with neutral chromatographic conditions using an Agilent 1290 UPLC system and Waters ACQUITY UPLC CSH C18 Column (130 Å, 1.7 µm, 2.1 mm X 150 mm). The column oven was set to 65 °C for both methods. The positive mode method had a flow rate of 0.4 mL minute^{-1} with mobile phase A consisting of 60:40 ACN/Water + 10 mM ammonium formate + 0.1% formic acid and B consisting of 90:10 IPA/ACN + 10 mM ammonium formate + 0.1% formic acid. The negative mode method had a flow rate of 0.4 mL minute^{-1} with mobile phase A consisting of 60:40 ACN/Water + 10 mM ammonium acetate and B consisting of 90:10 IPA/ACN + 10 mM ammonium acetate. Both methods used the same gradient of the following solvent B: 0.00 min 30%, 2.00 min 30%, 2.50 min 50%, 13.00 min 85%, 13.50 min 99%, 15.00 min 99%, 15.10 min 30% and 18.00 min 30%. Separated lipids were then ionised into the source of a Thermo Q-Exactive Plus mass spectrometer. Data were acquired via DDA topN, and scan range was set to 200–1200 m/*z*, with the only difference between the methods being the polarity (positive or negative mode). Every nine samples a blank and a quality check (QC) was injected.

Raw lipid spectral data (available at https://doi.org/https://doi.org/10.5281/zenodo.11560269) was exported to.raw files and processed in MS-Dial (version 5.2) (Tsugawa et al. 2015), for peak alignment, blank correction and lipid identification against the LipidMaps LipidFinder (V2) and Structure Database (LMSD). Any metabolites not matched were removed. Lipid values (representing the mass spectral area under the peak) for each sample were extracted from MS-Dial, and duplicate metabolites were assessed via match score (whereby the highest scoring lipid was retained) with any lipids with a total identification score of < 80% excluded (as per https://mtbinfo-team.github.io/mtbinfo.github.io/MS-DIAL/tutorial.html). Lipid data from positive and negative ion modes were combined, with any duplicate lipids filtered via match score as above. To compare the abundance of lipids between treatments (site and origin), all lipids were normalised to the peak area of an internal standard (negative: LPE 18:1 (d7), positive: TG 48:1 (d7)) resulting in a comparable relative value rather than an absolute quantity. The lipid abundance of coral eggs was normalised to the egg protein content (as per Matthews et al. 2023), while adult lipids were previously normalised to 50 ug protein biomass as described above, resulting in a relative lipid abundance comparable among treatments.

Due to the dissociation of wax esters using certain ionisation modes of mass spectrometry, identification can be challenging (Chen et al. 2015). Thus, given their known importance in marine organisms (Nevenzel 1970), a separate analysis was conducted to determine the wax ester composition of both the coral adults and eggs. Wax esters were extracted and analysed using the protocol as follows (Bosh and Long 2017): 30 mg of sample was extracted three times using 1 mL dichloromethane/methanol (DCM/MeOH) mixture (2:1) by vortexing for 3 min followed by sonication for 10 min. The combined extracts were dried under a stream of nitrogen gas and reconstituted in hexane. Tocopherol was used as the internal standard. Filtered hexane extracts were run on a GCMS-QP2020 (Shimadzu Corporation, Kyoto, Japan) equipped with an AOC-20is autosampler (Shimadzu Corporation). Samples were analysed on an SH-Rxi-5Sil MS fused silica capillary column (30.0 m × 0.25 mm × 0.25 µm) using the following settings (da Silva et al. 2020): electron impact mode at 70 eV; carrier gas—helium (1.0 mL minute^{-1}); injection volume -1 µL; injector temperature—280 °C; and ion source temperature – 200 °C. GC oven temperature settings was as follows: initial temperature 260 °C for 5 min, increased to 300 °C at 4 °C per minute and held for 17 min. Spectrum scan range was m/z 45–800. Metabolite identifications were performed by comparing mass spectral data using NIST-17 database (match score > 80%).

Statistical analysis

Egg size data were analysed via Welch's t test following satisfaction of normality, while egg count data did not meet assumptions and were analysed using a Wilcoxon rank sum test with a continuity correction. Differences in adult and egg protein concentrations were assessed using a two-way ANOVA (site and origin) and Welch's t test, respectively, as data met parametric assumptions. As the focus of this study was to understand specific changes in lipid species and classes rather than differences in the collective composition of lipids, an ANOVA approach was undertaken. Adult lipid relative abundances, including wax esters, were log transformed (with the addition of a small constant (0.000001) to prevent errors caused by log of 0; West 2022) and analysed using a two-way ANOVA where assumptions of normality (Shapiro–Wilk test) and homogeneity of variance (Bartlett test) were met, with site and origin as fixed factors, plus their interaction. Egg lipid relative abundances were similarly log transformed and analysed via a Welch's t test (where data were normal) by origin. Lipids that did not meet assumptions were instead analysed using an aligned ranks

transform (ART) ANOVA (Wobbrock et al. 2011) for adults and Wilcoxon rank sum test (eggs). Any lipid classes which contained ties (could not be accurately ranked due to equal values within the dataset) were subject to a permutation test (addition of small random values) with 10,000 permutations to confirm test outcomes. All tests were adjusted for multiple comparisons using a Benjamini–Hochberg false discovery rate. Statistical analyses were performed in R (R Core Team, 2021).

Results

Egg physical characteristics

No differences in egg count ($W = 28$, $p = 0.078$) were observed for nursery versus wild colonies, with an average of 4.6 (±0.2) and 5.5 (±0.4) eggs/bundle (mean ± SE) for nursery and wild colonies, respectively (Fig. 1). Similarly, egg size did not vary between eggs originating from nursery versus wild colonies, at 633.8 ± 12.4 (mean ± SE) and 621.9 ± 11.5 µm, respectively ($T_{40.8} = 0.682$, $p = 0.499$; Fig. 1).

Protein content

Adult tissue protein concentration did not vary between sites (Blue Lagoon vs Rayban), origin (nursery vs wild), or their combined interaction, with 0.39 (±0.03) and 0.41 (±0.05) mg cm ^{2} (mean ± SE) in nursery and wild colonies, respectively (Table S2, Fig. S2.A). Similarly, protein concentrations within *A. millepora* eggs did not differ between origin ($F_{12} = -1.010$, $p = 0.332$), with 0.34 ± 0.03 and 0.41 ± 0.05 mg (mean ± SE) for nursery and wild eggs, respectively (Fig. S2.B).

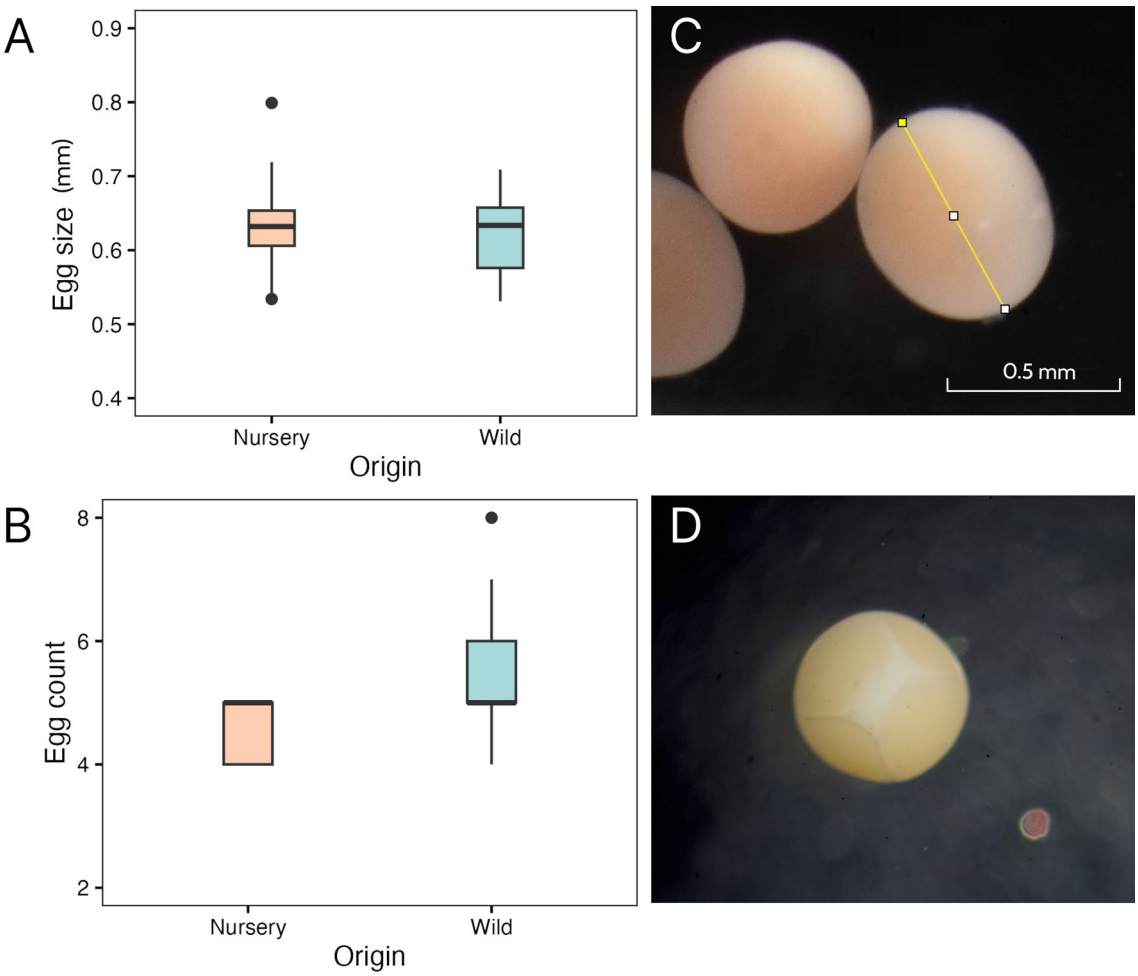

Fig. 1 **A** Egg size of *Acropora millepora* by origin from Blue Lagoon spawning November 2021 ($n = 23$ nursery, $n = 20$ wild), **B** Number of *A. millepora* eggs per egg-sperm bundle by origin from Blue Lagoon spawning 2021 ($n = 10$ per treatment), **C** Example length measurement taken for each *A. millepora* egg in ImageJ, **D** Photograph of *A. millepora* egg-sperm bundle with eggs still intact

Differences in adult lipid composition by site

Lipid composition of adult *A. millepora* colonies consisted of 28 identified lipid classes, with eight classes varying by site (i.e. Blue Lagoon vs Rayban; Table S2, Fig. 2). Blue Lagoon colonies were characterised by a significantly higher relative abundance in seven of the eight lipid classes (Table S2), with triacylglycerols ($F_1 = 40.840$, $p < 0.001$), phosphatidylcholines ($F_1 = 38.124$, $p < 0.001$) and lysophosphatidylcholines ($F_1 = 38.124$, $p < 0.001$) differing most significantly, while Rayban colonies were highest in a single lipid class, lysophosphatidylserine ($F_1 = 26.467$, $p < 0.001$). Within these lipid classes, 782 unique lipid species were identified in the adult corals, with 49 varying significantly between sites (Table S3). Blue Lagoon corals contained a greater relative abundance for 43 of the 49 lipid species (86%) found to differ between sites.

Differences in adult lipid composition by origin and site by origin interaction

When evaluated by propagule origin (nursery vs wild reef), 13 of the 28 lipid classes differed significantly. Lysophospholipid subclasses (lysophosphatidylcholines ($F_1 = 10.214$, $p = 0.004$), lysophosphatidylglycerol ($F_1 = 9.021$, $p = 0.008$), lysophosphatidylinositol ($F_1 = 16.165$, $p < 0.001$) and lysophosphatidylserine ($F_1 = 17.253$, $p < 0.001$)) and fatty acids (both saturated ($F_1 = 26.993$, $p < 0.001$) and unsaturated ($F_1 = 75.246$, $p < 0.001$)) were higher in abundance in wild colonies compared to nursery colonies, while sterol esters ($F_1 = 30.275$, $p < 0.001$), N-acylethanolamines ($F_1 = 5.877$, $p = 0.031$), phosphatidylcholines ($F_1 = 69.446$, $p < 0.001$), triacylglycerols ($F_1 = 114.729$, $p < 0.001$) and diacylglycerols ($F_1 = 26.270$, $p < 0.001$) were higher in nursery colonies (Table S3, Fig. 2). Site by origin interactions in adult colonies were also observed, whereby Blue Lagoon

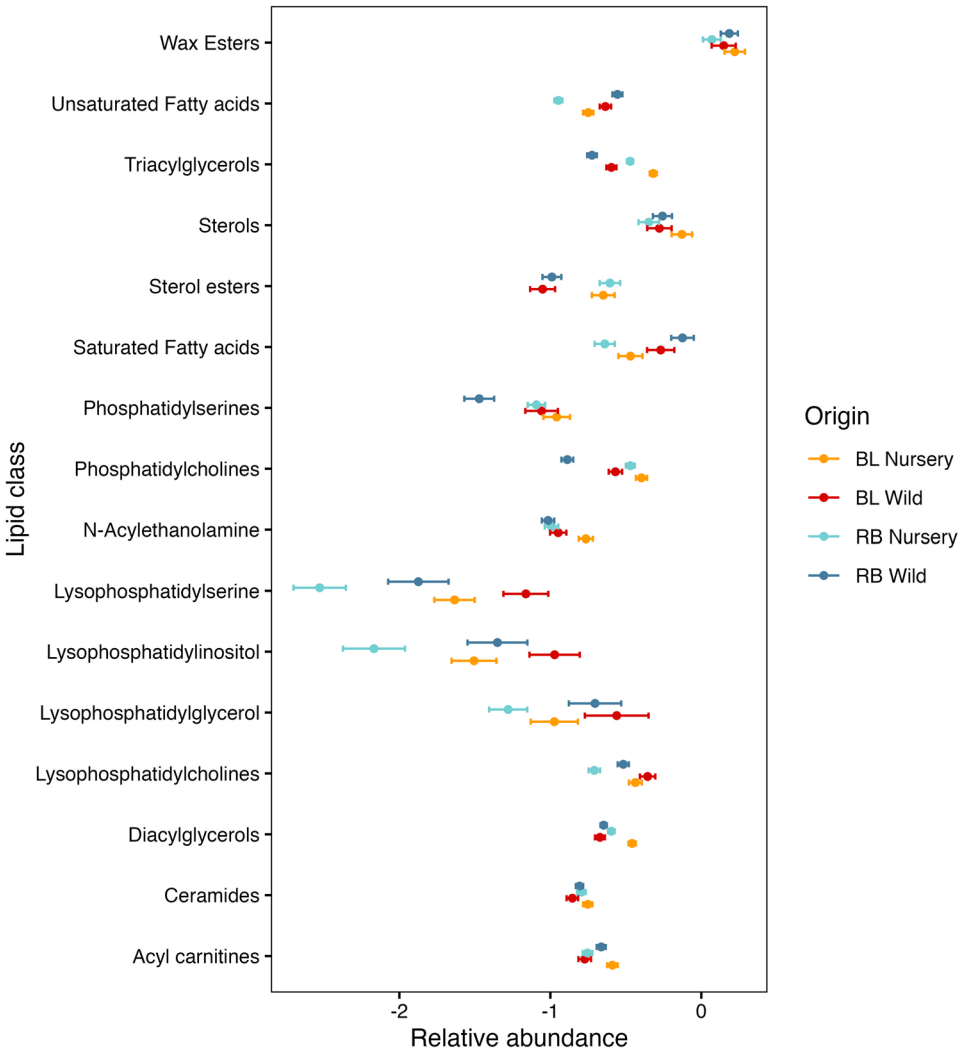

Fig. 2 Relative abundance of lipid classes (mean ± SE) from adult colonies of *Acropora millepora* by treatment (site—Blue Lagoon vs Rayban, and origin—nursery vs wild). BL = Blue Lagoon ($n = 11$), RB = Rayban ($n = 15$). Relative abundance has been log transformed

nursery colonies were significantly higher than Blue Lagoon wild colonies in acylcarnitines (Tukey's post hoc, $p < 0.001$) and diacylglycerols (Tukey's post hoc, $p < 0.001$), but this was not observed at Rayban. Additionally, Blue Lagoon and Rayban wild colonies had a significantly higher relative abundance of unsaturated fatty acids than their nursery counterparts within-site (Tukey's post hoc, $p = 0.044$, $p < 0.001$, respectively; Fig. 2). When separated into unique lipid species, no species varied by origin following the false discovery rate correction (Benjamini–Hochberg method), although 32 lipid species were significantly different prior to this correction. Only one lipid species was found to differ in abundance between the interaction of origin and site ($F_1 = 13.604$, $p = 0.001$; Table S3), with the wax ester tetradecanoic acid hexadecyl ester in greater abundance in Rayban wild corals compared to Rayban nursery colonies (Tukey's post hoc, $p = 0.007$; Table S4).

Egg lipid composition by origin

Within the identified 32 lipid classes, eggs originating from Blue Lagoon nursery colonies during the 2021 spawning event had a significantly higher abundance of ceramide ($W = 1{,}638{,}070$, $p = 0.002$), triacylglycerol ($W = 2{,}073{,}130$, $p < 0.001$) and phosphatidylcholine ($W = 6{,}582{,}435$, $p < 0.001$) classes (Table S5, Fig. 3), while wild eggs had a greater abundance of wax esters ($T_{39} = -2.178$, $p = 0.036$). Over 2,270 unique lipid species were identified within the *A. millepora* eggs (Table S6), with the only difference in lipid species abundance found for five identified wax ester compounds (Table S5). Hexadecanoic acid hexadecyl ester, hexadecyl pentadecanoate, lauric acid tridecyl ester, decanoic acid tetradecyl ester and decanoic acid tridecyl ester were found in higher abundance in eggs originating from wild versus nursery colonies (Table S6).

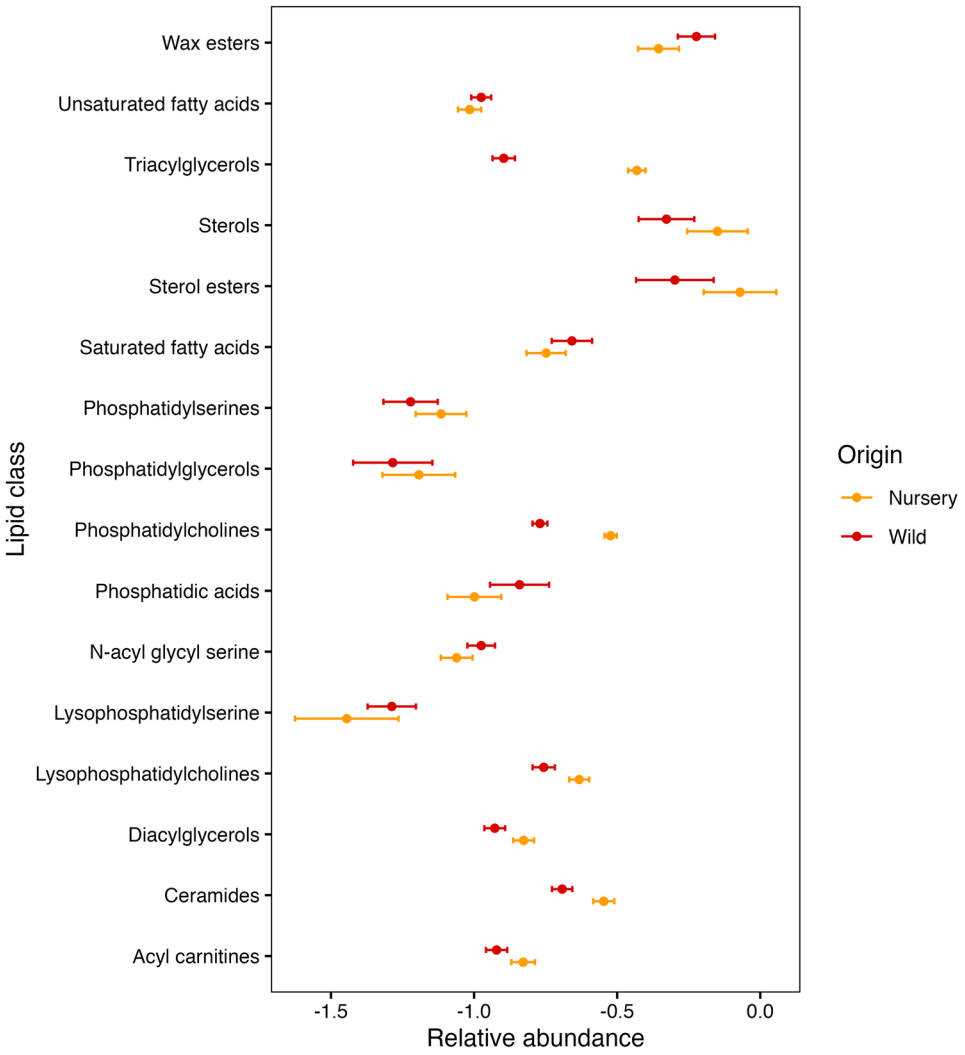

Fig. 3 Relative abundance of lipid classes (mean ± SE) from *Acropora millepora* eggs by origin (nursery vs wild) from Blue Lagoon ($n = 6$ nursery, $n = 7$ wild). Relative abundance has been log transformed

Discussion

Nursery-based coral propagation has grown in popularity worldwide, including on the Great Barrier Reef (McLeod et al. 2022). We therefore examined patterns in lipid composition, protein content and physical egg characteristics (egg size and count) to investigate the influence of propagation environment on key physiological indicators of adult corals and their eggs. Differences in lipid profiles were found for adult colonies between sites (Blue Lagoon vs Rayban), as well as for adult corals and their eggs across origins (nursery versus native reef). Adult nursery colonies were richer in storage lipids and lipid classes responsible for regulating membrane structure (i.e. phosphatidylcholines and sterol esters), while wild adult corals were characterised by elevated levels of fatty acids and classes involved in immunoregulation (lysophospholipids). In comparison, egg lipid profiles from nursery eggs had elevated stress-regulating ceramides, phosphatidylcholines important for cell membrane structure and energy-storing triacylglycerols, whereas wild eggs had a greater abundance of wax ester species. Notably, patterns in egg lipid profiles did not mirror those of adult colonies from the same origin, suggesting that parental effects may have lesser influence on lipid composition and that environmental conditions could drive differences across coral life stages. In contrast, egg size and count did not vary with origin, and tissue protein content of adult colonies and their eggs also did not differ across reef sites (adults) or origin (adults and eggs). Characterising fitness indicators—beyond those commonly used in restoration, i.e. growth and survival—of coral propagules reared under distinct conditions (i.e. on nursery platforms) provides insight into the influence of propagation practices on restoration material.

Differences in adult lipid profiles between origins

Our current study supports previous findings that propagation origin influences the energetic profiles of adult colonies (Nuñez Lendo et al. in press), as selected lipids were in higher abundance in nursery corals and others more abundant in wild colonies (summarised in Table S1.B). Nursery colonies were characterised by elevated levels of sterol esters, phosphatidylcholines and tri/diacylglycerols. Sterol esters play a role in membrane homeostasis and accumulation of free sterols (Ferrer et al. 2017; Korber et al. 2017), but more specifically have been highlighted for their role in biomineralisation of the coral skeleton (Farre et al. 2010). High abundances of key energy lipids (triacylglycerols and diacylglycerols) and structural/biomineralisation lipids responsible for fuelling cellular activities and required for growth and calcification are consistent with previous hypotheses that nursery corals may experience favourable conditions compared to adjacent reef environments. Previous research conducted at the same coral nurseries has observed that growth rates, photosynthesis, symbiont density, respiration and calcification were higher in *A. hyacinthus* nursery colonies compared to wild corals (Nuñez Lendo et al. in press). Thus, when considered alongside our observations of elevated energy and biomineralisation lipids, this may suggest that nurseries support greater resource acquisition (though heterotrophic or autotrophic sources) or reduced exposure to negative biotic or abiotic stressors such as competition, corallivory or sedimentation which can divert resources to defensive responses in an effort to maintain fitness (Herlan and Lirman 2008; Rinkevich 2019; Shafir et al. 2006a). Although we hypothesise on potential factors which could be influencing lipid profiles, greater characterisation of environmental factors is ultimately needed to resolve these disparities in lipid patterns across propagation origins.

Wild corals were richer in several bioactive and signalling lysophospholipids (lysophosphatidylcholines, lysophosphatidylglycerol, lysophosphatidylinositol and lysophosphatidylserine), alongside saturated and unsaturated fatty acids. Lysophosphatidylcholine and lysophosphatidylserine are known to have anti-inflammatory and immunoregulatory effects (Frasch and Bratton 2012; Lin et al. 2023; Stien et al. 2020; Tan et al. 2020), suggesting that wild corals may have upregulated responses of proinflammatory lipids to help minimise influences of environmental stress, such as the higher mean and maximum temperatures experienced by the reef environment compared to nurseries at Blue Lagoon (Table S1B). Fatty acids are the building blocks of lipids and serve a diversity of functions including membrane structure, energy supply and immune function (Calder 2015; De Carvalho and Caramujo 2018; Radice et al. 2019). Unsaturated fatty acids (UFAs) have a lower melting point compared to saturated fatty acids (SFAs; Liu et al. 2018), with elevated concentrations linked to greater stability in membrane fluidity, thereby promoting thermostability of corals under environmental stress (Tchernov et al. 2004; von Xylander et al. 2023). As such, the ratio between SFAs and UFAs is a widely used metric for evaluating membrane stability under oxidative stress since UFAs are more susceptible to oxidation (Dubousquet et al. 2016; Kostetsky et al. 2018; Liu et al. 2020, 2022). Wild colonies exhibited a higher ratio of saturated-to-unsaturated fatty acids (SFA/UFA) compared to nursery colonies (2.28 vs 1.70, respectively), which may reflect declines in UFAs (due to possible peroxidation) driven by the elevated temperatures experienced on the reef and the need to maintain optimal membrane fluidity. Fatty acids can also serve as a metabolic source of energy among other diverse functions (Radice et al. 2019), potentially suggesting strategies of energy storage, utilisation or feeding ecology may differ among nursery and wild colonies (Grottoli et al. 2004). While

specific fatty acid molecules are often used as dietary markers to indicate differences in trophic energy sources in corals, such as from Symbiodiniaceae, diatoms, copepods or algal sources (Figueiredo et al. 2012), a lack of disparity in fatty acid species between origins (i.e. corals from both origins exhibited the same diversity of fatty acids) suggests that broader dietary sources may not have differed significantly, but rather the overall synthesis or catabolysis of fatty acid classes potentially due to differences in metabolic processes or requirements.

Differences in egg lipid profiles between origins

Egg lipid composition varied by source origin, with ceramides, phosphatidylcholines and triacylglycerols higher in nursery-reared eggs, while wax esters were higher in eggs originating from wild colonies (Table S1B). Wax esters play an essential role in coral egg buoyancy and dispersal, constituting a long-term energy source in marine organisms (Arai et al. 1993; Harii et al. 2007; Lee et al. 2006). Conversely, triacylglycerols are believed to be consumed more rapidly, satisfying short-term energy requirements (Lee et al. 2006; Lin et al. 2013). Thus, our findings could suggest energy provisioning strategies differ between parents from nursery versus wild origins as a reflection of different propagation environments that in turn could influence the dispersal range and development rates of their offspring. Such results therefore intriguingly suggest that nursery offspring will have shorter dispersal ranges and fertilisation time due to reduced buoyancy and long-term energy provisioning, whereas wild eggs could have reduced energy necessary for rapid cell-division during early development (Figueiredo et al. 2012; Okubo et al. 2020). However, examining ontogenetic changes in the reef-building coral *Goniastrea retiformis* has shown wax esters declined significantly over a 30-d period relative to other lipids, suggesting that wax esters may be important for short-term development in coral larvae (Figueiredo et al. 2012). By contrast, ceramides and phosphatidylcholines have been noted for their importance in corals for structural integrity, environmental stress-regulation and maintenance of membrane fluidity under extreme conditions (Figueiredo et al. 2012; Lin et al. 2012, 2013), indicating that environmental factors may be driving increases in the investment of structural lipids more heavily in nursery eggs compared to wild eggs. Our work captures the lipid profiles of *A. millepora* eggs at a single site, and thus, additional work is needed to elucidate these various hypotheses of energy and stress lipid functional roles across different sites, coral species, life stages and life-history strategies, such as those offspring which are provisioned with symbionts prior to release.

Patterns in adult lipid composition were not reflected in eggs

Comparison of Blue Lagoon adult and egg lipid profiles provided insight into patterns in lipid composition across coral life stages for this species. Interestingly, apart from similarities in storage lipids, whereby adult nursery colonies had high levels of diacylglycerols that may have contributed to the high synthesis of triacylglycerols observed in the eggs (Bay et al. 2013), patterns in lipid composition exhibited by nursery and wild adult colonies were not reflected in eggs from the same origin at Blue Lagoon (Table S1.B). A previous study that examined coral lipid profiles showed that adults and their offspring differed in their biochemical composition (Padilla-Gamino et al. 2013). While parent *Montipora capitata* corals displayed high phenotypic variation in lipid composition across environments, these disparities were not displayed in their offspring, suggesting that provisioned energy reserves are relatively conserved in coral eggs (Padilla-Gamino et al. 2013). As such, source origin could be more important for driving lipid profiles in coral eggs than parental effects, highlighting the need to consider a range of life stages when examining the influence of restoration practices on coral fitness and population resistance.

A. millepora eggs were composed of a considerably greater diversity of lipid species compared to their adult colonies (2,270 versus 782, respectively). Lipids are the primary biochemical constituent of coral eggs (Figueiredo et al. 2012; Harii et al. 2007; Harland et al. 1993) and are known to be present in higher concentrations in eggs compared to adults (Padilla-Gamino et al. 2013). As little work has been undertaken on the untargeted characterisation of the coral egg lipidome to date, limited knowledge exists to explain our finding that *A. millepora* eggs contained nearly three times the number of lipid species as adult colonies. However, the lecithotrophic nature of many coral larvae (although likely not exclusively lecithotrophic, as corals have shown capacity to feed exogenously; Graham et al. 2013; Harii et al. 2007; Rivest et al. 2017; Rodd et al. 2022) requires that coral offspring be provisioned with the quantity and quality of lipids necessary to sustain dispersal and growth demands, nutritional requirements, membrane formation and signalling processes during early development. As such, it could be posited that coral eggs require a greater diversity of lipid species compared to adult corals to ensure their survival during sensitive life stages and highlight an area in need of greater understanding. Our work characterising the lipidome of coral eggs provides a good first step in understanding the diversity of lipids necessary for egg fitness and survival, but that clearly warrants more detailed study.

Similarities in protein content and egg physical characteristics across sites and origins

Alongside energetic profiles of coral offspring, physical egg characteristics can provide insight on reproductive fitness as a reflection of parental investment (Bellworthy et al. 2019; Michalek-Wagner and Willis 2001). The uniform sizes observed for wild versus nursery eggs indicates resource investment by parent colonies were conserved across the two environments whereby the total volume of energetic material (i.e. lipids and proteins) provisioned to offspring did not differ. When considered alongside recent work demonstrating increased growth in adult nursery colonies of another *Acropora* species (*A. hyacinthus*) from the same nurseries (Nuñez Lendo et al. in press), we would hypothesise that egg volume could be reduced in our nursery colonies given the greater energy budget directed towards growth rather that reproduction. However, such an outcome was not observed, with *A. millepora* nursery colonies appearing to retain the same proportion of reproductive investment in their offspring as wild colonies, suggesting that nursery propagation yields no negative impacts to sexually produced propagules in regard to provisioning volume. Future work should examine these traits across species and whether documented growth enhancements in nursery colonies carry over to differences in physiological responses in future life stages such as settlement and survival of larvae.

Protein analysis also revealed neither adult corals nor their offspring differed in their tissue protein concentration between nursery and wild colonies. These results are again consistent with a previous study examining traits of nursery-propagated corals on the same nursery platforms, whereby increased growth and calcification of *A. hyacinthus* was observed, but this was not accompanied by a greater abundance of total protein (Nuñez Lendo et al. in press). Thus, if *in situ* nurseries are providing energetic benefits to colonies (for example, through greater provisioning of nutrients or planktonic material, enhanced autotrophy, or a combination), these energetic benefits are being synthesised directly into tissue growth and skeletal extension, rather than being stored as reserve in their tissue biomass. Ultimately, our results show that the protein content remains largely consistent between nursery and reef environments, suggesting that conditions do not differ enough to impact the overall protein abundance in these tissues.

Conclusion

In summary, patterns in coral adult and egg lipid classes support the notion that nursery colonies may be experiencing favourable conditions for energy acquisition or reduced environmental and/or biological stressors diverting resources, demonstrated by elevated abundances of key energy and biomineralisation lipid classes. Coral eggs did not reflect the same patterns as adult colonies and could indicate that source origin may exert a greater influence on coral energetics compared to parental effects. Our work captures the lipid profiles of small sample size of coral adults and eggs of a single species, and thus, additional work is needed to support our findings across other taxa and reefs. Nonetheless, this research contributes to the growing body of work supporting the use of omics analysis as a sensitive and early response indicator (Abdelkader et al. 2023) and highlights the need to look beyond traditional fitness responses of growth and survival to assess restoration effects. Future work should examine how egg lipid profiles translate to differences in larval rigour (e.g. dispersal distance, settlement success, survivorship) to assess whether biological trade-offs are occurring. Understanding the influence of restoration practices on propagule energetic status is necessary for providing a viable means of increasing coral biomass yields which underpin healthy and resilience reef populations into the future.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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