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Biochemical Differentiation of Freshly Isolated and Free-Living Cultured *Symbiodinium microadriaticum*: Fatty Acid Signatures and Proximate Composition

Andrea Martino [1,2,*], Valerio Isa [3,4], Davide Seveso [4,5,6], Francisco Otero-Ferrer [7], Davide Maggioni [4,6,8], Francesco Saliu [5], Daniel Montero [2], Paolo Galli [4,5,6], Silvia Lavorano [1] and Francesco Cicala [9,*]

1 Costa Edutainment S.p.A.—Acquario di Genova, Area Porto Antico, Ponte Spinola, 16128 Genoa, Italy
2 Aquaculture Research Group (IU-ECOAQUA), University of Las Palmas de Gran Canaria, Carretera de Taliarte, s/n, 35200 Telde, Spain
3 Center of Nanoscience and Technology, Italian Institute of Technology, 20133 Milan, Italy
4 MaRHE Center (Marine Research and High Education Center), Magoodhoo Island 12030, Faafu Atoll, Maldives
5 Department of Earth and Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan, Italy
6 National Biodiversity Future Center (NBFC), 90133 Palermo, Italy
7 BIOCON (IU-ECOAQUA), University of Las Palmas de Gran Canaria, Carretera de Taliarte, s/n, 35200 Telde, Spain
8 Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy
9 Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy
* Correspondence: andrea.martino101@alu.ulpgc.es (A.M.); francesco.cicala@unipd.it (F.C.)

Abstract

Coral-associated dinoflagellates undergo substantial physiological changes when transferred from the host environment to laboratory culture, yet direct compositional comparisons remain limited. We compared the proximate composition and fatty acid profiles of *Symbiodinium microadriaticum* (ITS2 type A1) freshly isolated from *Stylophora pistillata* with free-living cultures derived from the same coral-associated symbiont material and maintained under controlled conditions. Total lipid and crude protein contents did not differ significantly between states, whereas fatty acid composition differed markedly. Sixteen individual fatty acids remained significantly different after Benjamini-Hochberg false discovery rate correction. Freshly isolated samples showed higher relative abundances of several saturated, monounsaturated, and n-6 polyunsaturated fatty acids, including 16:0, 18:0, 16:1n-7, arachidonic acid, and docosatetraenoic acid, together with a higher proportion of total n-6 fatty acids. Free-living cells showed higher relative abundances of 14:0, 16:3n-4, α-linolenic acid, and 18:5n-3, together with higher proportions of total PUFA, n-3, and n-9 fatty acids and a higher docosahexaenoic acid/arachidonic acid (DHA/ARA) ratio. These contrasting signatures indicate marked state-associated biochemical differentiation despite similar bulk lipid and protein contents. The results provide a direct compositional reference for interpreting cultured Symbiodiniaceae used in algal physiology, coral-algal symbiosis research, and experimental host acquisition.

Keywords: Symbiodiniaceae; coral-algal symbiosis; dinoflagellate physiology; fatty acids; lipid metabolism; *Stylophora pistillata*

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1. Introduction

Coral reefs are among the most diverse and productive ecosystems on Earth, despite persisting in nutrient-poor tropical waters [1,2]. Their ecological success largely depends on mutualistic associations with intracellular dinoflagellates of the family Symbiodiniaceae, commonly referred to as zooxanthellae [3,4]. These photosynthetic symbionts provide a substantial fraction of the host's metabolic energy through the translocation of photosynthetically fixed carbon, thereby supporting host metabolism, calcification, and reef growth [5]. In addition to carbohydrates, Symbiodiniaceae contribute to host lipid pools, including polyunsaturated fatty acids (PUFAs), highlighting the importance of lipid metabolism within the coral holobiont [5–8]. Conversely, conditions established by the host can influence symbiont metabolism and nutrient allocation. This metabolic interdependence is vulnerable to environmental disturbances that destabilize the association and can ultimately contribute to coral bleaching [9–11].

Among Symbiodiniaceae, *Symbiodinium microadriaticum* is widely used as a model for investigating cnidarian and dinoflagellate symbiosis [12,13]. Within the host, symbiont cells occur predominantly in a coccoid, non-motile state and reside inside host-derived symbiosomes that regulate molecular and nutritional exchanges between partners [4,14–17]. The symbiosome constitutes a highly regulated intracellular environment that differs from external seawater in nutrient availability, inorganic carbon chemistry, light exposure, and other physicochemical conditions. Host-nutrient regulation, particularly nitrogen limitation, can restrict symbiont proliferation and modify carbon allocation and storage metabolism [17,18].

Transition to ex hospite cultivation exposes Symbiodiniaceae to a different nutritional and physicochemical environment and can therefore be accompanied by substantial physiological changes. Earlier comparisons between freshly isolated and cultured symbionts documented differences in morphology, protein expression, carbon allocation, and lipid storage [19–22]. Moreover, freshly isolated and long-term cultured *Symbiodinium* A1 cultures were shown to differ in photophysiological performance, growth, neutral lipid accumulation, and thylakoid lipid unsaturation, further demonstrating that cultivation history can influence the biochemical phenotype of symbiotic dinoflagellates [23]. These observations emphasize that cultured Symbiodiniaceae should not automatically be considered biochemically equivalent to their *in hospite* counterparts.

Recent studies have further expanded our understanding of lipid plasticity within Symbiodiniaceae. Comprehensive lipidomic analyses demonstrated extensive, species-specific remodeling of membrane and storage lipid pools in response to thermal stress [24]. In cultured *Durusdinium glynnii*, irradiance was shown to modify growth, pigment production, and fatty acid composition [25], while cultivation and inoculation strategies affected both growth performance and fatty acid allocation during scale-up [26]. Together, these studies demonstrate that Symbiodiniaceae lipid metabolism is highly responsive to physiological and environmental context. However, comparative information directly assessing the fatty acid composition of coral-associated and cultured *S. microadriaticum* remains scarce.

This distinction is relevant to the biology of cnidarian and dinoflagellate symbiosis because fatty acids participate in both structural and energetic processes and may contribute to metabolic exchange between symbiotic partners [6–8]. Lipid composition in Symbiodiniaceae has been associated with physiological performance and thermal tolerance, while symbiont-derived fatty acids can contribute to host lipid pools and nutritional status [6–8,24,27,28]. Characterizing biochemical differences between freshly isolated and cultured cells may therefore provide information that cannot be inferred from taxonomic identity or cell density alone, and may improve interpretation of cultured Symbiodiniaceae used in experimental host recolonization and assisted symbiont acquisition [29,30].

Accordingly, the present study compared the biochemical profile of *S. microadriaticum* under two biologically distinct conditions: (i) symbiont cells isolated from *Stylophora pistillata*, representing the freshly isolated condition, and (ii) coral-derived cells maintained as free-living cultures under controlled laboratory conditions. We hypothesized that these conditions would be associated with distinct patterns of fatty acid allocation, potentially reflecting their integrated nutritional and metabolic characteristics. By combining molecular characterization, proximate composition, and fatty acid profiling, this study aimed to provide a biochemical framework for interpreting differentiation between freshly isolated and cultured *S. microadriaticum*.

2. Materials and Methods

2.1. Coral Husbandry and Symbiont Isolation

Coral maintenance and symbiont isolation were carried out at the Tropical Department of the Aquarium of Genoa (Genoa, Italy). Adult donor colonies of *S. pistillata* were maintained under controlled conditions in recirculating aquaculture systems (RAS). Temperature was maintained at 25 ± 1 °C, salinity at 35 ± 1‰, and pH at 8.3 ± 0.1, under a 12 h light:12 h dark photoperiod. Incident irradiance measured in the immediate vicinity of the coral colonies was approximately 150 µmol photons m^{-2} s^{-1}. *S. pistillata* was used as the source host because its association with *S. microadriaticum* ITS2 A1 has been reported in natural and experimental holobionts [31,32]. Moreover, previous ITS2 sequencing of *S. pistillata* mother colonies from the same Aquarium stock identified *Symbiodinium* as the predominant symbiont genus [33].

The experimental workflow is summarized in Figure 1. Symbiont isolation was carried out from six independent *S. pistillata* fragments (~7 cm), each collected from a different donor colony and processed separately using a low-stress calcium- and magnesium-free seawater (CMFSW) detachment protocol adapted from Isa et al. [30]. Briefly, coral fragments were incubated in CMFSW for 3 h to promote symbiont release while preserving cellular integrity and minimizing host-derived contamination [30]. Following incubation, the cell suspension was filtered through a 20 µm nylon mesh to remove large fragments of coral tissue and skeletal debris and obtain a symbiont preparation. Released symbiont cells were then concentrated by low speed centrifugation (800× *g* for 5 min) using a Rotofix 32A benchtop centrifuge (Hettich, Kirchlengern, Germany). Immediately after isolation, aliquots of each coral-derived symbiont suspension were examined by bright-field microscopy (Leica, Microsystem GmbH, Wetzlar, Germany) to document cell integrity and general morphology and were counted using a Neubauer haemocytometer. Representative freshly isolated cells are shown in Figure 2A. This procedure generated six independent symbiont pellets. Three pellets were processed immediately as freshly isolated samples (n = 3) for biochemical analyses. For these samples, the algal biomass was briefly rinsed with deionized water to minimize residual salts, lyophilized, and stored at −80 °C until analysis. The other three pellets were used to establish three independent free-living cultures.

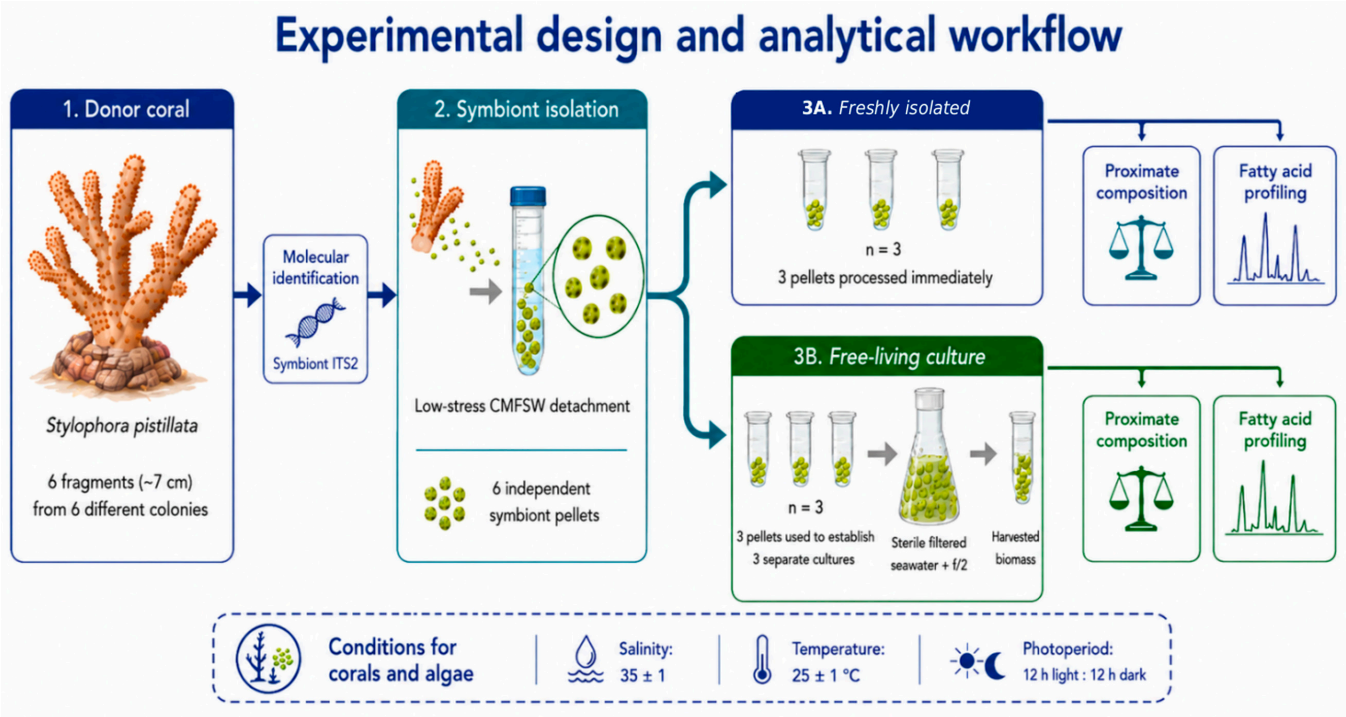


Figure 1. Experimental design used to compare freshly isolated and free-living *Symbiodinium microadriaticum*. Six independent *Stylophora pistillata* fragments were processed separately for symbiont isolation. Three independently obtained algal pellets were analyzed immediately as freshly isolated samples, while three additional pellets were used to establish independent free-living cultures. Molecular identification was performed on source coral material, whereas each biological replicate was processed for proximate composition and fatty acid profiling.

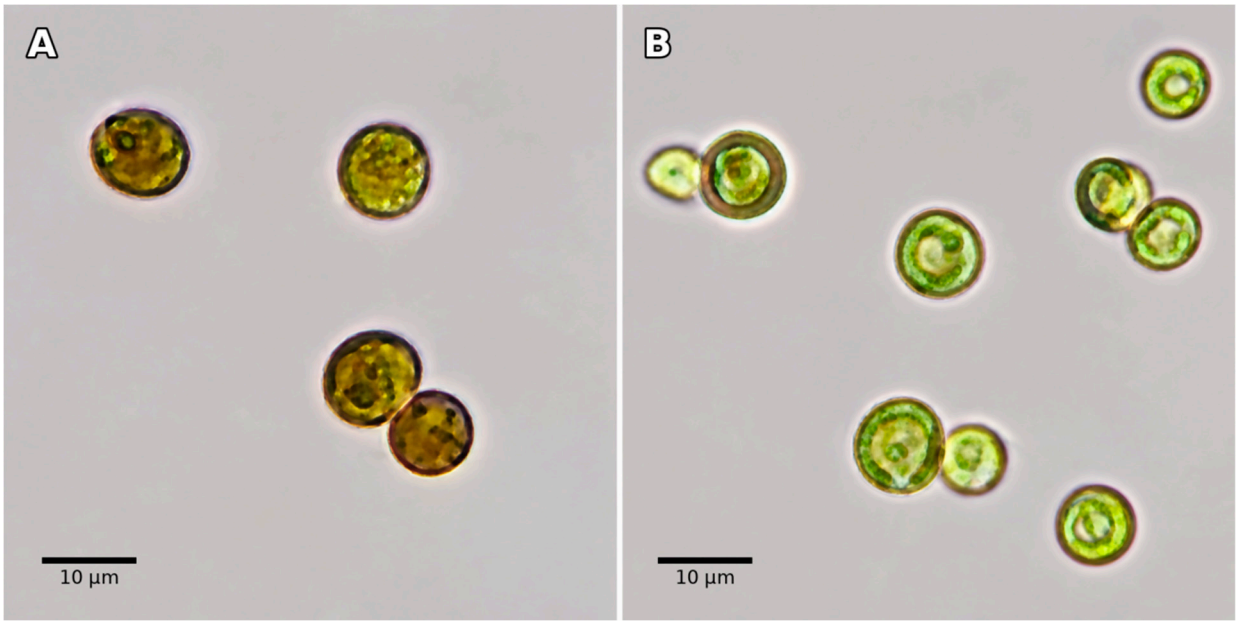


Figure 2. Representative micrographs of coral-derived *Symbiodinium microadriaticum* preparations obtained from molecularly characterized *Stylophora pistillata* colonies. (**A**) Freshly isolated cells immediately after separation from coral tissue; (**B**) cultured cells at harvest. Both preparations were dominated by coccoid, pigmented cells showing a well-defined peripheral boundary and peripheral chloroplast-associated pigmentation. Reddish-brown intracellular inclusions compatible with accumulation bodies are visible in some cells. Scale bars = 10 µm.

2.2. Establishment and Harvesting of Free-Living Cultures

The three pellets of *S. microadriaticum* assigned to culture were maintained as independent biological replicates (n = 3) throughout the experiment (Figure 1). Cultures were grown in sterile filtered f/2 medium without silicate, according to Guillard and Ryther [34]. Seawater was 0.2 µm filtered, UV sterilized, and autoclaved before use. Salinity and temperature were maintained at 35 ± 1‰ and 25 ± 1 °C, respectively, under a 12 h light:12 h dark photoperiod. Illumination was provided by LED lights (LEDX GmbH, Ganserndorf, Austria) at a photon flux density of 150 µmol photons m^{-2} s^{-1}, measured at the culture surface. Cultures were maintained in sterile borosilicate Erlenmeyer flasks and manually mixed once daily. Initial inoculations were performed in 50 mL flasks, after which cultures were progressively scaled up to a final volume of 5 L over 30 days. During scaleup, cultures were diluted with fresh f/2 medium to maintain active growth. During culture establishment, scale-up, and harvesting, cell integrity, general morphology, culture homogeneity, and cell concentration were monitored by bright-field microscopy (Leica, Germany) and using a Neubauer haemocytometer. No morphologically distinct microalgal contaminants were observed. Representative cultured cells at harvest are shown in Figure 2B. Molecular characterization was performed on the source coral-associated material as described in Section 2.3. All cultures were harvested during the exponential growth phase and at the same time of day to minimize variability in biochemical composition. Biomass was recovered by low speed centrifugation using a Rotofix 32A bench-top centrifuge (Hettich, Germany). The harvested biomass was briefly rinsed with deionized water to minimize residual medium salts, lyophilized, frozen at −80 °C, and stored until proximate biochemical and fatty acid analyses.

2.3. Molecular Characterization of the Coral Host and Symbiont

At the beginning of the study, molecular analyses were performed at the University of Padua (Padua, Italy) on source coral fragments to confirm host identity and characterize the predominant associated Symbiodiniaceae ITS2 sequence before the freshly isolated and cultured preparations were compared. Small coral fragments (~2 cm) were preserved in molecular-grade ethanol (96%) and used for total genomic DNA extraction, yielding bulk holobiont DNA containing both host and associated symbiont DNA. Total genomic DNA was extracted from ethanol preserved coral fragments using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's instructions. The same DNA extracts were used for marker-specific amplification of the host cytochrome c oxidase subunit I (COI) region and the symbiont Internal Transcribed Spacer 2 (ITS2) region. The predominant Symbiodiniaceae ITS2 sequence was characterized by PCR amplification using the primers SYM_VAR_5.8S2 and SYM_VAR_REV [35]. For *S. pistillata* identification, a portion of the COI gene was amplified using the primers LCO1490 and HCO2198 [36]. PCR reactions were performed in 25 µL final volumes containing 5 µL of Phusion High-Fidelity PCR Master Mix 2× (Thermo Fisher Scientific, Waltham, MA, USA), 2 µL of each primer (10 µM), 2.5 µL of template DNA, and 13.5 µL of nuclease-free water. The thermal profile consisted of an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 10 s, 46 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. PCR amplification was verified by electrophoresis on a 1.5% agarose gel. Amplicons were bidirectionally Sanger sequenced using an ABI 3730xl DNA Analyser (Applied Biosystems, Waltham, MA, USA). Raw sequences were edited using FinchTV (Geospiza Inc., v1.4.0, Denver, CO, USA) and aligned using MEGA 11 [37]. Consensus sequences were queried against the NCBI database using BLASTn (BLAST+ v2.17.0) for taxonomic identification.

2.4. Proximate and Fatty Acid Composition Analyses

Proximate biochemical characterization and fatty acid profiling were conducted at the laboratories of the Highly Specialized Aquaculture and Biotechnology Service (SABE) located at the University Institute for Sustainable Aquaculture and Marine Ecosystems (IU-ECOAQUA), University of Las Palmas de Gran Canaria, following analytical procedures previously applied at the same facility for the proximate and fatty acid characterization of marine biological samples [38]. All proximate biochemical and fatty acid determinations were performed in technical triplicate; for each biological replicate, the mean of technical replicates was used for statistical analyses.

Proximate biochemical composition was quantified in freshly isolated and free-living cultured *S. microadriaticum* samples and values were expressed as g/100 g DW. Samples were briefly rinsed with deionized water to minimize residual salts and then lyophilized to determine dry weight (DW). Moisture content was determined by thermal dehydration at 105 °C until constant weight according to AOAC procedures [39]. Crude protein was determined using the Kjeldahl method and calculated as N × 6.25 [39]. Total lipids were extracted gravimetrically from lyophilized biomass using the chloroform:methanol method described by Folch et al. [40].

Fatty acid composition was determined from total lipid extracts obtained from each biological replicate of the freshly isolated and cultured samples. Fatty acid methyl esters (FAMEs) were prepared by acid-catalyzed transmethylation of aliquots of the total lipid extracts with 1% (*v*/*v*) sulfuric acid in methanol, following Christie and Han [41]. The reaction was conducted under a nitrogen atmosphere, protected from light, for 16 h at 50 °C. After cooling, FAMEs were recovered by extraction with hexane:diethyl ether (1:1, *v*/*v*), and the organic phase was concentrated under nitrogen before gas-chromatographic analysis. FAMEs were separated using a 7820A GC System (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame-ionization detector and a Supelcowax-10 fused-silica capillary column (30 m × 0.32 mm i.d.; Supelco, Bellefonte, PA, USA), using helium as the carrier gas. The column temperature was maintained at 180 °C for 10 min, increased to 215 °C at 2.5 °C min^{-1}, and maintained at 215 °C for 10 min, following the chromatographic conditions described by Izquierdo et al. [42]. Individual FAMEs were identified by comparison of retention times with the well-characterized EPA 28 fish-oil reference (Nippai Ltd., Tokyo, Japan) analysed under the same chromatographic conditions. Fatty acid composition was calculated from integrated peak areas and expressed as relative abundance of total fatty acids (% total FA), with each biological replicate normalized to 100%. Fatty acid summations were calculated from the relative abundance data, and selected fatty acid ratios were calculated from the corresponding individual fatty acid values.

2.5. Statistical Analysis

All results are expressed as mean ± standard deviation (SD) of three independent biological replicates per condition, obtained from six independently processed coral-derived symbiont preparations. For biochemical analyses conducted in technical triplicate, technical values were first averaged within each biological replicate, and replicate level means were used for statistical analysis. Differences in proximate composition between free-living and freshly isolated states were evaluated using two-tailed Welch's *t*-tests, which do not assume homogeneity of variances. Degrees of freedom were estimated using the Welch-Satterthwaite approximation. Statistical significance was set at $p < 0.05$.

Individual fatty acid relative abundances were compared between freshly isolated and free-living conditions using two-tailed Welch's *t*-tests. Given the large number of parallel comparisons, *p* values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) procedure, and FDR-adjusted values < 0.05 were considered statistically significant.

Variables showing no variation across all samples were not subjected to inferential testing. Fatty acid summations (ΣSAFA, ΣMUFA, ΣPUFA, Σn-3, Σn-6, Σn-9, and ΣLC-PUFA n-3) and selected fatty acid ratios (DHA/EPA, DHA/ARA, and EPA/ARA) were compared between conditions using Welch's *t*-tests without further correction, as these were predefined composite fatty acid indices rather than exploratory individual variables.

Fatty acid profile structure was explored descriptively using principal component analysis (PCA) on standardized fatty acid relative-abundance data (% total FA). Variables were mean-centered and scaled to unit variance, and PCA was computed based on the correlation matrix. Dashed grouping circles were included in the score plot to visualize replicate dispersion within each condition. All statistical analyses, including PCA, were performed using IBM SPSS Statistics v27.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Molecular Identification

Molecular analyses confirmed the taxonomic identity of the coral host and characterized the predominant coral-associated Symbiodiniaceae ITS2 sequence before biochemical comparisons were performed. PCR amplification of the mitochondrial COI region from bulk DNA extracts obtained from ethanol-preserved coral fragments generated amplicons suitable for Sanger sequencing. Subsequent sequence comparison against reference databases confirmed the host as *S. pistillata*. Top matches showed 98–100% query coverage, sequence identities up to 99.85%, and E-value of 0.0, confirming the taxonomic identity of the analyzed coral colonies. No nucleotide substitutions were detected among individual coral sequences, indicating that all analyzed colonies shared the same mitochondrial lineage. BLASTn analysis of ITS2 consensus sequences obtained from the same coral-derived DNA extracts showed the highest similarity to *S. microadriaticum* sequences deposited in the NCBI database. Top matches showed 100% query coverage, sequence identities up to 98.23%, and an E-value of 4×10^{-135}, identifying *S. microadriaticum* ITS2 type A1 [43] as the predominant ITS2 sequence recovered from the source colonies. Alignment of individual ITS2 sequences revealed two closely related haplotypes differing by four nucleotide substitutions. However, this limited sequence variability was not investigated further, as it was beyond the scope of the present study.

3.2. Microscopic Observations

Bright-field microscopy documented predominantly coccoid, pigmented cells in both freshly isolated suspensions and cultures at harvest (Figure 2A,B), consistent with the morphology described for Symbiodinium [12,14,17]. Cells in both preparations showed a well-defined peripheral boundary and peripheral pigment distribution compatible with the cell covering and reticulate chloroplast organization of the coccoid stage [12,14]. A reddish-brown intracellular inclusion compatible with the accumulation body was visible. Freshly isolated cells appeared generally darker and larger, whereas cultured cells appeared more lightly pigmented and visually smaller, a qualitative pattern consistent with previous comparisons of freshly isolated and cultured Symbiodiniaceae [21]. The cultured preparations remained morphologically homogeneous, and no morphologically distinct microalgal contaminants were observed during routine monitoring. Cell concentrations were approximately 8.0×10^5 cells mL^{-1} in freshly isolated suspensions and 4.0×10^6 cells mL^{-1} in cultures at harvest.

3.3. Proximate Composition

No statistically significant differences in proximate composition were detected between free-living and freshly isolated cells ($p > 0.05$; Figure 3). Although total lipid content was

numerically higher in the freshly isolated condition, reaching 12.0 ± 0.3 g/100 g DW, compared with 11.1 ± 0.7 g/100 g DW in free-living cultures (Figure 3), this difference was not statistically significant (Welch's t = −2.08, df = 2.57, p = 0.144). Crude protein content was also numerically higher in freshly isolated samples than in free-living cells, with values of 48.7 ± 1.2 g/100 g DW and 43.2 ± 2.9 g/100 g DW, respectively (Figure 3), although the difference was not statistically significant (Welch's t = −3.02, df = 2.69, p = 0.065).

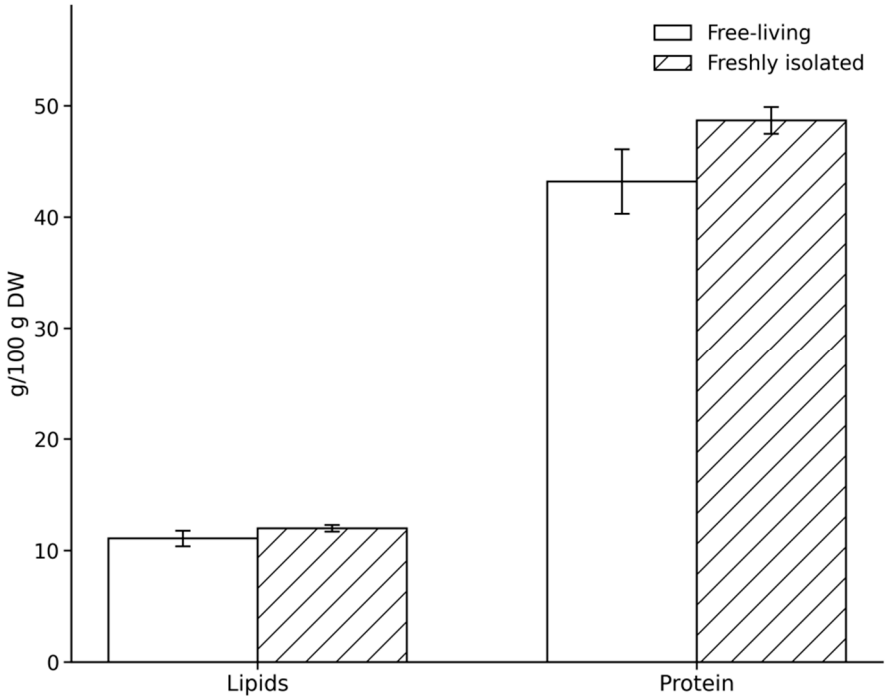


Figure 3. Proximate composition of free-living and freshly isolated *Symbiodinium microadriaticum*. Values are expressed as mean ± SD (n = 3 independent biological replicates) and reported as g/100 g dry weight (DW). Open bars indicate free-living samples, while hatched bars indicate freshly isolated samples. Differences between physiological states were evaluated using Welch's t-test.

3.4. Fatty Acid Composition

Marked differences in fatty acid relative abundance were observed between free-living and freshly isolated cells (Table 1; Figure 4). After Benjamini-Hochberg FDR correction, 16 individual fatty acids remained significantly different between conditions (FDR < 0.05). Freshly isolated cells showed higher relative abundances of 16:0 (28.96 ± 1.93 vs. 18.97 ± 0.20%), 16:1n-7 (7.58 ± 0.68 vs. 3.55 ± 0.01%), 17:0 (0.43 ± 0.06 vs. 0.07 ± 0.02%), 18:0 (16.12 ± 2.29 vs. 1.45 ± 0.17%), 20:1n-7 (1.61 ± 0.12 vs. 0.21 ± 0.00%), 20:1n-5 (0.33 ± 0.03 vs. 0.04 ± 0.01%), 20:2n-6 (0.28 ± 0.05 vs. 0.10 ± 0.02%), arachidonic acid (ARA; 20:4n-6; 2.52 ± 0.37 vs. 0.16 ± 0.04%), and docosatetraenoic acid (DTA; 22:4n-6; 1.49 ± 0.23 vs. 0.09 ± 0.02%). Conversely, free-living cells showed higher relative abundances of 14:0 (23.67 ± 0.79 vs. 2.40 ± 0.27%), 15:0 (1.91 ± 0.26 vs. 1.05 ± 0.27%), 16:3n-4 (0.33 ± 0.06 vs. 0.10 ± 0.04%), 18:1n-7 (2.97 ± 0.08 vs. 2.62 ± 0.10%), α-linolenic acid (ALA; 18:3n-3; 5.11 ± 0.16 vs. 0.17 ± 0.03%), 22:5n-6 (0.50 ± 0.08 vs. 0.05 ± 0.02%), and 18:5n-3 (9.33 ± 0.75 vs. 4.77 ± 0.93%) (Table 1; Figure 4).

Principal component analysis provided an exploratory visualization of the overall fatty acid profiles (Figure 5). PC1 and PC2 explained 64.6% and 16.5% of the total variance, respectively, accounting together for 81.0% of total variation. Samples clustered according to physiological condition primarily along PC1.

Table 1. Proximate composition and individual fatty acid relative abundance in free-living and freshly isolated *Symbiodinium microadriaticum*. Values are expressed as mean ± SD (n = 3 independent biological replicates). Total lipids and crude protein are reported as g/100 g dry weight (DW), whereas individual fatty acids are expressed as percentage of total fatty acids (% total FA). Differences between physiological states were evaluated using two-tailed Welch's t-tests. For individual fatty acids, p values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) procedure. Asterisks indicate FDR < 0.05. Statistical analyses were performed on unrounded replicate-level values. FDR, false discovery rate; n.a., not applicable.

Parameter	Free-Living	Freshly Isolated	Welch t	df	p Value	FDR	FDR < 0.05
Total lipids	11.1 ± 0.7	12.0 ± 0.3	−2.08	2.57	0.144	n.a.	n.a.
Crude protein	43.2 ± 2.9	48.7 ± 1.2	−3.02	2.69	0.065	n.a.	n.a.
14:0	23.67 ± 0.79	2.40 ± 0.27	44.183	2.457	<0.001	0.006	*
14:1n-7	0.05 ± 0.00	0.18 ± 0.12	−1.826	2.000	0.209	0.287	
14:1n-5	0.19 ± 0.04	0.23 ± 0.01	−1.423	2.493	0.267	0.320	
15:0	1.91 ± 0.26	1.05 ± 0.27	4.024	3.991	0.016	0.048	*
15:1n-5	0.06 ± 0.03	0.14 ± 0.09	−1.534	2.508	0.240	0.303	
16:0 ISO	0.13 ± 0.07	0.05 ± 0.02	1.946	2.228	0.178	0.267	
16:0	18.97 ± 0.20	28.96 ± 1.93	−8.908	2.041	0.012	0.040	*
16:1n-7	3.55 ± 0.01	7.58 ± 0.68	−10.258	2.002	0.009	0.037	*
16:1n-5	0.22 ± 0.15	0.07 ± 0.02	1.666	2.067	0.234	0.303	
16:2n-6	0.09 ± 0.07	0.04 ± 0.04	1.107	3.252	0.343	0.374	
16:2n-4	0.33 ± 0.20	0.18 ± 0.06	1.296	2.339	0.308	0.344	
17:0	0.07 ± 0.02	0.43 ± 0.06	−9.718	2.346	0.006	0.037	*
16:3n-4	0.33 ± 0.06	0.10 ± 0.04	5.415	3.418	0.009	0.037	*
16:3n-3	0.04 ± 0.02	0.06 ± 0.04	−0.531	3.066	0.631	0.631	
16:3n-1	0.12 ± 0.08	1.64 ± 0.77	−3.406	2.039	0.074	0.149	
16:4n-3	0.05 ± 0.06	3.63 ± 1.24	−5.002	2.011	0.037	0.090	
18:0	1.45 ± 0.17	16.12 ± 2.29	−11.071	2.022	0.008	0.037	*
18:1n-9	17.56 ± 0.21	9.91 ± 2.41	5.478	2.029	0.031	0.080	
18:1n-7	2.97 ± 0.08	2.62 ± 0.10	4.664	3.812	0.011	0.040	*
18:1n-5	0.10 ± 0.02	0.08 ± 0.04	0.788	3.166	0.486	0.496	
18:2n-9	0.07 ± 0.02	0.17 ± 0.04	−4.376	3.152	0.020	0.056	
18:2n-6	4.59 ± 0.26	2.42 ± 1.17	3.118	2.202	0.079	0.152	
18:2n-4	0.02 ± 0.01	0.02 ± 0.00	−1.540	3.571	0.207	0.287	
18:3n-6	0.24 ± 0.07	0.63 ± 0.31	−2.163	2.183	0.152	0.244	
18:3n-4	0.04 ± 0.01	0.05 ± 0.01	−2.076	3.967	0.107	0.183	
18:3n-3	5.11 ± 0.16	0.17 ± 0.03	53.279	2.105	<0.001	0.006	*
18:3n-1	0.01 ± 0.01	0.30 ± 0.16	−3.007	2.007	0.095	0.168	
18:4n-3	3.55 ± 0.17	2.74 ± 1.02	1.359	2.108	0.301	0.344	
18:4n-1	0.06 ± 0.01	0.07 ± 0.02	−1.019	2.728	0.390	0.416	
18:5n-3	9.33 ± 0.75	4.77 ± 0.93	6.622	3.831	0.003	0.030	*
20:0	0.49 ± 0.32	1.09 ± 0.13	−2.965	2.608	0.071	0.149	
20:1n-9	0.51 ± 0.12	0.36 ± 0.10	1.651	3.862	0.177	0.267	
20:1n-7	0.21 ± 0.00	1.61 ± 0.12	−20.926	2.003	0.002	0.027	*
20:1n-5	0.04 ± 0.01	0.33 ± 0.03	−16.804	2.485	0.001	0.020	*
20:2n-9	0.04 ± 0.00	0.07 ± 0.03	−1.730	2.044	0.223	0.297	
20:2n-6	0.10 ± 0.02	0.28 ± 0.05	−6.289	2.499	0.014	0.044	*
20:3n-9	0.04 ± 0.02	0.06 ± 0.00	−1.843	2.052	0.203	0.287	
20:3n-6	0.05 ± 0.02	1.00 ± 0.47	−3.476	2.011	0.073	0.149	
20:4n-6	0.16 ± 0.04	2.52 ± 0.37	−10.868	2.041	0.008	0.037	*
20:3n-3	0.31 ± 0.26	0.18 ± 0.07	0.841	2.272	0.480	0.496	
20:4n-3	0.12 ± 0.07	0.54 ± 0.32	−2.237	2.170	0.145	0.240	
20:5n-3	0.55 ± 0.18	1.00 ± 0.29	−2.355	3.357	0.091	0.167	
22:1n-11	0.07 ± 0.04	0.16 ± 0.04	−3.238	4.000	0.032	0.080	
22:1n-9	0.17 ± 0.07	0.24 ± 0.03	−1.476	2.702	0.246	0.303	

Table 1. *Cont.*

Parameter	Free-Living	Freshly Isolated	Welch t	df	*p* Value	FDR	FDR < 0.05
22:4n-6	0.09 ± 0.02	1.49 ± 0.23	−10.616	2.032	0.008	0.037	*
22:5n-6	0.50 ± 0.08	0.05 ± 0.02	10.060	2.294	0.006	0.037	*
22:5n-3	0.10 ± 0.03	0.23 ± 0.06	−3.405	3.149	0.039	0.090	
22:6n-3	1.57 ± 0.20	1.95 ± 0.44	−1.358	2.798	0.273	0.320	

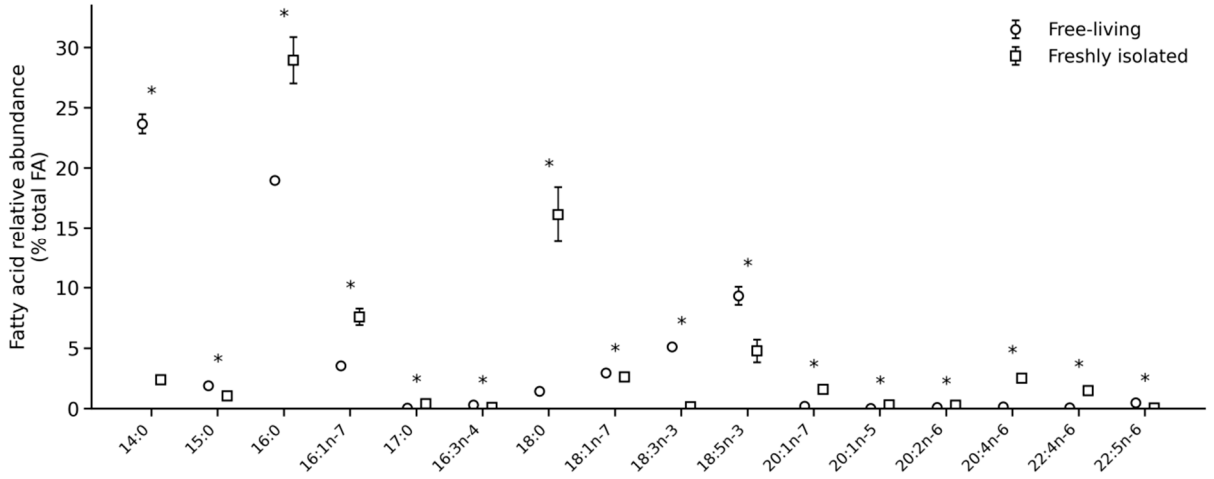


Figure 4. Key individual fatty acids differing between free-living and freshly isolated *Symbiodinium microadriaticum*. Values are expressed as mean ± SD (n = 3 independent biological replicates) and reported as percentage of total fatty acids (% total FA). Open circles indicate free-living samples, while open squares indicate freshly isolated samples. The figure includes the 16 fatty acids that remained significant after Benjamini-Hochberg false discovery rate correction (FDR < 0.05). Asterisks indicate significant differences after FDR correction.

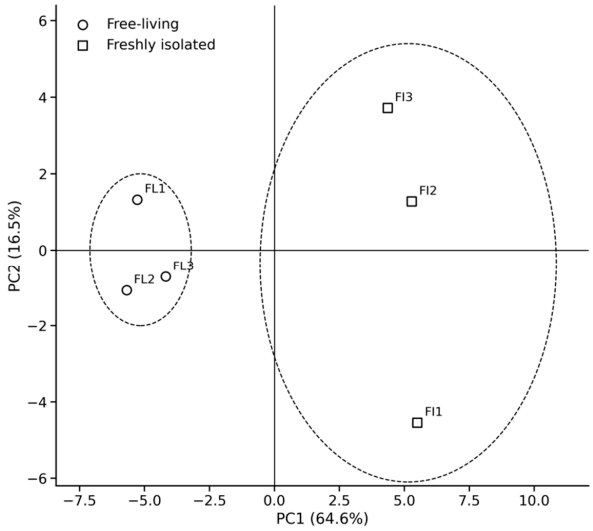


Figure 5. Principal component analysis (PCA) of standardized fatty acid composition data in free-living and freshly isolated *Symbiodinium microadriaticum*. PCA was performed on fatty acid relative abundances expressed as percentage of total fatty acids (% total FA), after mean-centering and scaling to unit variance. PC1 and PC2 explained 64.6% and 16.5% of total variance, respectively. Open circles indicate free-living samples, while open squares indicate freshly isolated samples. Dashed grouping circles visualize replicate dispersion within each physiological state.

3.5. Fatty Acid Summations and Ratios

Fatty acid summations further highlighted compositional differences between physiological states (Table 2). ΣPUFA and Σn-3 were significantly higher in free-living cells (27.61 ± 0.48% and 20.73 ± 0.55%, respectively) than freshly isolated cells (26.38 ± 0.54% and 15.27 ± 1.23%; Welch's t = 2.944, p = 0.043 and Welch's t = 7.040, p = 0.008, respectively). Σn-9 was also significantly higher in free-living cells (18.39 ± 0.27%) than in freshly isolated cells (10.82 ± 2.46%; Welch's t = 5.291, p = 0.032). Conversely, Σn-6 was significantly higher in freshly isolated cells (8.44 ± 0.70%) than in free-living cells (5.82 ± 0.19%; Welch's t = −6.278, p = 0.017). ΣSAFA, ΣMUFA, and ΣLC-PUFA n-3 did not differ significantly between conditions. The DHA/ARA ratio remained significantly higher in free-living cells (10.19 ± 3.20) than in freshly isolated cells (0.77 ± 0.15; Welch's t = 5.092, p = 0.036), whereas DHA/EPA and EPA/ARA did not differ significantly between conditions (p = 0.104 and p = 0.078, respectively; Table 2).

Table 2. Fatty acid summations and selected fatty acid ratios in free-living and freshly isolated *Symbiodinium microadriaticum*. Summations are expressed as mean ± SD (n = 3 independent biological replicates) and reported as percentage of total fatty acids (% total FA), whereas ratios are dimensionless. Differences between physiological states were evaluated using two-tailed Welch's t-tests. Asterisks indicate significant differences at $p < 0.05$. ΣSAFA, saturated fatty acids; ΣMUFA, monounsaturated fatty acids; ΣPUFA, polyunsaturated fatty acids; ΣLC-PUFA n-3, long-chain n-3 polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid.

Parameter	Free-Living	Freshly Isolated	Welch t	df	p Value	$p < 0.05$
ΣSAFA	46.69 ± 0.52	50.11 ± 1.99	−2.878	2.268	0.089	
ΣMUFA	25.70 ± 0.37	23.52 ± 2.52	1.485	2.084	0.271	
ΣPUFA	27.61 ± 0.48	26.38 ± 0.54	2.944	3.948	0.043	*
Σn-3	20.73 ± 0.55	15.27 ± 1.23	7.040	2.763	0.008	*
Σn-6	5.82 ± 0.19	8.44 ± 0.70	−6.278	2.302	0.017	*
Σn-9	18.39 ± 0.27	10.82 ± 2.46	5.291	2.049	0.032	*
ΣLC-PUFA n-3	2.65 ± 0.17	3.90 ± 1.07	−2.013	2.103	0.175	
DHA/EPA	3.00 ± 0.65	1.96 ± 0.13	2.717	2.154	0.104	
EPA/ARA	3.63 ± 1.67	0.40 ± 0.09	3.347	2.011	0.078	
DHA/ARA	10.19 ± 3.20	0.77 ± 0.15	5.092	2.009	0.036	*

4. Discussion

The present study shows that freshly isolated and cultured *S. microadriaticum* differed primarily in fatty acid composition, whereas no statistically significant differences were detected in total lipid or crude protein content. This pattern was supported by FDR-adjusted differences in 16 individual fatty acids and by differences in selected composite indices, while PCA provided an exploratory representation of overall profile structure. These findings extend previous evidence that transition from the host environment to culture can alter biochemical allocation in Symbiodiniaceae [19–22]. The present comparison demonstrates that similar bulk lipid contents can coexist with pronounced differences in the relative distribution of individual fatty acids.

Freshly isolated cells were characterized by higher relative abundances of 16:0, 16:1n-7, 17:0, 18:0, 20:1n-7, 20:1n-5, 20:2n-6, ARA, and DTA, together with a higher Σn-6. This pattern was selective rather than representative of a general enrichment in saturated or monounsaturated fatty acids, because ΣSAFA and ΣMUFA did not differ significantly between conditions. The intracellular environment provides a biologically plausible context for this differentiation. Symbionts *in hospite* experience host-regulated nutrient availability and physicochemical conditions that differ substantially from nutrient-replete culture medium. In particular, nitrogen availability regulates symbiont proliferation, carbon

allocation, and life-history characteristics, and nitrogen-limited *S. microadriaticum* cultures can display molecular and physiological features resembling those observed *in hospite* [17]. The higher relative contribution of ARA and DTA, together with the higher Σn-6, was among the most distinctive components of the freshly isolated profile. Previous studies have detected Symbiodiniaceae-associated PUFAs in coral host tissues [6] and patterns consistent with the transfer of host-derived PUFAs to symbionts [7]. However, the present results do not establish the direction of fatty acid transfer or the functional role of individual n-6 fatty acids. Recent lipidomic evidence shows that Symbiodiniaceae can reorganize specific membrane and storage lipid classes in response to physiological conditions [24].

Free-living cultured cells showed a contrasting profile characterized by higher relative abundances of 14:0, 15:0, 16:3n-4, 18:1n-7, ALA, 22:5n-6, and particularly 18:5n-3. Consistently, ΣPUFA, Σn-3, and Σn-9 were higher in cultured cells. The high abundance of 18:5n-3 highlights the strong contribution of C18 n-3 PUFA to the free-living profile, whereas ΣLC-PUFA n-3 did not differ significantly between conditions, indicating that the difference in total n-3 fatty acids was driven predominantly by shorter-chain components rather than by EPA, DPA n-3, or DHA. The DHA/ARA ratio was also higher in cultured cells; because DHA itself did not differ significantly between conditions, this difference was driven primarily by the markedly lower relative abundance of ARA. The higher relative abundance of 16:3n-4, ALA, and 18:5n-3 therefore indicates a distinct allocation of C16 and C18 PUFAs under laboratory cultivation. More broadly, fatty acid composition in cultured dinoflagellates is sensitive to cultivation history, irradiance, salinity, and culture-management conditions [23,25,26,44–46]. The occurrence of 18:5n-3 in the present profiles is also consistent with previous reports for *S. microadriaticum* [45].

ITS2 sequencing was performed on the source coral-associated material and was not repeated on the cultures at harvest. The predominance of *S. microadriaticum* ITS2 type A1 at isolation, together with the morphological homogeneity observed throughout cultivation, supports continuity of the cultured preparations; nevertheless, selective enrichment of a minority, morphologically similar Symbiodiniaceae lineage during culture establishment cannot be entirely excluded. For the DW-based proximate measurements, residual inorganic material cannot be completely excluded despite filtration and brief rinsing, and the deionized-water rinse may have induced some osmotic stress or cell lysis. Because fatty acid data are expressed as relative proportions of the fatty acid pool, differences should be interpreted as compositional redistribution rather than as changes in absolute fatty acid concentration. Reporting fatty acids as % total FA provides an integrated assessment of fatty acid composition but does not resolve their distribution among membrane, storage, or signaling lipid classes [47]. Within this analytical scope, the results nevertheless demonstrate a clear compositional distinction between freshly isolated and cultured cells.

PCA was used as an exploratory representation of profile structure and interpreted alongside the FDR-adjusted univariate comparisons. The profiles generated here provide a compositional reference for interpreting cultured Symbiodiniaceae in physiological studies and experiments involving symbiont acquisition [29,30]. In particular, they identify the individual fatty acids and composite indices contributing most strongly to the biochemical distinction between freshly isolated and cultured *S. microadriaticum*. Future studies combining fatty acid profiling with lipid-class separation, lipidomics, and stable-isotope tracing could further clarify how specific lipid pools change outside the host and how these changes relate to nutrient exchange and metabolic integration within the coral-algal association.

5. Conclusions

Coral-derived *S. microadriaticum* preparations analyzed immediately after isolation and after free-living culture exhibited distinct fatty acid profiles, whereas no statistically

significant differences were detected in total lipid or crude protein content. Sixteen individual fatty acids remained significantly different after FDR correction, demonstrating that biochemical differentiation between the two conditions was expressed primarily through redistribution within the fatty acid pool. Free-living cells showed higher contributions of total PUFA, n-3, and n-9 fatty acids, including a higher abundance of 18:5n-3, whereas freshly isolated cells showed a higher contribution of n-6 fatty acids and higher ARA and DTA.

The direct comparison of coral-derived cells analysed immediately after isolation with free-living cultures provides a compositional baseline for evaluating biochemical variation between host-associated and cultured conditions. These profiles may support interpretation of experiments employing cultured Symbiodiniaceae, particularly in studies of symbiont physiology and experimental host acquisition, while emphasizing that similar bulk lipid and protein contents can coexist with pronounced differences in fatty acid composition.

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Data Availability Statement: Representative Sanger sequences used for molecular characterization of the source material have been deposited in GenBank under accession numbers PZ477849 (*S. microadriaticum* ITS2) and PZ477851 (*S. pistillata* COI). The raw datasets supporting the biochemical and statistical analyses are available from the corresponding authors on reasonable request.

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References

1. Hatcher, B.G. Coral reef primary productivity: A beggar's banquet. *Trends Ecol. Evol.* **1988**, *3*, 106–111. [CrossRef] [PubMed]
2. Allgeier, J.E. The ecosystem ecology of coral reefs revisited. *Annu. Rev. Ecol. Evol. Syst.* **2024**, *55*, 251–370. [CrossRef]
3. Muscatine, L.; Porter, J.W. Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. *BioScience* **1977**, *27*, 454–460. [CrossRef]
4. Roth, M.S. The engine of the reef: Photobiology of the coral-algal symbiosis. *Front. Microbiol.* **2014**, *5*, 422. [CrossRef] [PubMed]

5. Yellowlees, D.; Rees, T.A.V.; Leggat, W. Metabolic interactions between algal symbionts and invertebrate hosts. *Plant Cell Environ.* **2008**, *31*, 679–694. [CrossRef] [PubMed]
6. Papina, M.; Meziane, T.; Van Woesik, R. Symbiotic zooxanthellae provide the host-coral *Montipora digitata* with polyunsaturated fatty acids. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* **2003**, *135*, 533–537. [CrossRef] [PubMed]
7. Imbs, A.B.; Yakovleva, I.M.; Dautova, T.N.; Bui, L.H.; Jones, P. Diversity of fatty acid composition of symbiotic dinoflagellates in corals: Evidence for the transfer of host PUFAs to the symbionts. *Phytochemistry* **2014**, *101*, 76–82. [CrossRef] [PubMed]
8. Chen, H.K.; Wang, L.H.; Chen, W.N.U.; Mayfield, A.B.; Levy, O.; Lin, C.S.; Chen, C.S. Coral lipid bodies as the relay center interconnecting diel-dependent lipidomic changes in different cellular compartments. *Sci. Rep.* **2017**, *7*, 3244. [CrossRef] [PubMed]
9. Baker, A.C.; Glynn, P.W.; Riegl, B. Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuar. Coast. Shelf Sci.* **2008**, *80*, 435–471. [CrossRef]
10. Wiedenmann, J.; D'Angelo, C.; Smith, E.G.; Hunt, A.N.; Legiret, F.E.; Postle, A.D.; Achterberg, E.P. Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nat. Clim. Change* **2013**, *3*, 160–164. [CrossRef]
11. Hughes, T.P.; Kerry, J.T.; Connolly, S.R.; Baird, A.H.; Eakin, C.M.; Heron, S.F.; Hoey, A.S.; Hoogenboom, M.O.; Jacobson, M.; Liu, G.; et al. Ecological memory modifies the cumulative impact of recurrent climate extremes. *Nat. Clim. Change* **2019**, *9*, 40–43. [CrossRef]
12. Freudenthal, H.D. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: Taxonomy, life cycle, and morphology. *J. Protozool.* **1962**, *9*, 45–52. [CrossRef]
13. LaJeunesse, T.C.; Parkinson, J.E.; Gabrielson, P.W.; Jeong, H.J.; Reimer, J.D.; Voolstra, C.R.; Santos, S.R. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr. Biol.* **2018**, *28*, 2570–2580.e6. [CrossRef] [PubMed]
14. Trench, R.K.; Blank, R.J. *Symbiodinium microadriaticum* Freudenthal, *S. goreauii* sp. nov., *S. kawagutii* sp. nov. and *S. pilosum* sp. nov.: Gymnodinioid dinoflagellate symbionts of marine invertebrates. *J. Phycol.* **1987**, *23*, 469–481. [CrossRef]
15. Davy, S.K.; Allemand, D.; Weis, V.M. Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol. Mol. Biol. Rev.* **2012**, *76*, 229–261. [CrossRef] [PubMed]
16. Aranda, M.; Li, Y.; Liew, Y.J.; Baumgarten, S.; Simakov, O.; Wilson, M.C.; Piel, J.; Ashoor, H.; Bougouffa, S.; Bajic, V.B.; et al. Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. *Sci. Rep.* **2016**, *6*, 39734. [CrossRef] [PubMed]
17. Cui, G.; Liew, Y.J.; Konciute, M.K.; Zhan, Y.; Hung, S.H.; Thistle, J.; Gastoldi, L.; Schmidt-Roach, S.; Dekker, J.; Aranda, M. Nutritional control regulates symbiont proliferation and life history in coral-dinoflagellate symbiosis. *BMC Biol.* **2022**, *20*, 103. [CrossRef] [PubMed]
18. Jiang, P.L.; Pasaribu, B.; Chen, C.S. Nitrogen-deprivation elevates lipid levels in *Symbiodinium* spp. by lipid droplet accumulation: Morphological and compositional analyses. *PLoS ONE* **2014**, *9*, e87416. [CrossRef] [PubMed]
19. Peng, S.E.; Chen, C.S.; Song, Y.F.; Huang, H.T.; Jiang, P.L.; Chen, W.N.U.; Fang, L.S.; Lee, Y.C. Assessment of metabolic modulation in free-living versus endosymbiotic *Symbiodinium* using synchrotron radiation-based infrared microspectroscopy. *Biol. Lett.* **2012**, *8*, 434–437. [CrossRef] [PubMed]
20. Chen, H.K.; Song, S.N.; Wang, L.H.; Mayfield, A.B.; Chen, Y.J.; Chen, W.N.U.; Chen, C.S. A compartmental comparison of major lipid species in a coral–*Symbiodinium* endosymbiosis: Evidence that the coral host regulates lipogenesis of its cytosolic lipid bodies. *PLoS ONE* **2015**, *10*, e0132519. [CrossRef] [PubMed]
21. Pasaribu, B.; Weng, L.C.; Lin, I.P.; Camargo, E.; Tzen, J.T.C.; Tsai, C.H.; Ho, S.L.; Lin, M.R.; Wang, L.H.; Chen, C.S.; et al. Morphological variability and distinct protein profiles of cultured and endosymbiotic *Symbiodinium* cells isolated from *Exaiptasia pulchella*. *Sci. Rep.* **2015**, *5*, 15353. [CrossRef] [PubMed]
22. Wang, L.H.; Chen, H.K.; Jhu, C.S.; Cheng, J.O.; Fang, L.S.; Chen, C.S. Different strategies of energy storage in cultured and freshly isolated *Symbiodinium* sp. *J. Phycol.* **2015**, *51*, 1127–1136. [CrossRef] [PubMed]
23. Arossa, S.; Klein, S.G.; Alva Garcia, J.V.; Steckbauer, A.; Pluma, N.; Genchi, L.; Laptenok, S.P.; Hung, S.H.; Salazar, O.R.; Aranda, M.; et al. Differential responses to heat stress between freshly isolated and long-term cultured *Symbiodinium*. *Microorganisms* **2026**, *14*, 455. [CrossRef] [PubMed]
24. Botana, M.T.; Chaves-Filho, A.B.; Inague, A.; Güth, A.Z.; Saldanha-Corrêa, F.; Müller, M.N.; Sumida, P.Y.G.; Miyamoto, S.; Kellermann, M.Y.; Valentine, R.C.; et al. Thermal plasticity of coral reef symbionts is linked to major alterations in their lipidome composition. *Limnol. Oceanogr.* **2022**, *67*, 1456–1469. [CrossRef]
25. Oliveira, C.Y.B.; Abreu, J.L.; Santos, E.P.; Matos, Â.P.; Tribuzi, G.; Oliveira, C.D.L.; Veras, B.O.; Bezerra, R.S.; Müller, M.N.; Gálvez, A.O. Light induces peridinin and docosahexaenoic acid accumulation in the dinoflagellate *Durusdinium glynnii*. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 6263–6276. [CrossRef] [PubMed]
26. de Sena, P.R.; Lira, M.E.S.S.; Oliveira, D.W.S.; Brandão, B.d.C.S.; de Abreu, J.L.; Gama, W.A.; Araújo, E.S.; Tribuzi, G.; Gálvez, A.O.; Oliveira, C.Y.B. Scale-up cultivation of the dinoflagellate *Durusdinium glynnii* under varying inoculum percentages: Effects on growth performance and fatty acid profile. *Phycology* **2025**, *5*, 21. [CrossRef]

27. Díaz-Almeyda, E.; Thomé, P.E.; El Hafidi, M.; Iglesias-Prieto, R. Differential stability of photosynthetic membranes and fatty acid composition at elevated temperature in *Symbiodinium*. *Coral Reefs* **2011**, *30*, 217–225. [CrossRef]
28. Rosset, S.; Koster, G.; Brandsma, J.; Hunt, A.N.; Postle, A.D.; D'Angelo, C. Lipidome analysis of Symbiodiniaceae reveals possible mechanisms of heat stress tolerance in reef coral symbionts. *Coral Reefs* **2019**, *38*, 1241–1253. [CrossRef]
29. Scharfenstein, H.J.; Chan, W.Y.; Buerger, P.; Humphrey, C.; van Oppen, M.J.H. Evidence for de novo acquisition of microalgal symbionts by bleached adult corals. *ISME J.* **2022**, *16*, 1676–1679. [CrossRef] [PubMed]
30. Isa, V.; Martino, A.; Montalbetti, E.; Toniolo, L.M.; Dalla Rovere, G.; Casati, A.; Seveso, D.; Louis, Y.D.; Saliu, F.; Galli, P.; et al. Early experimental comparison of natural and assisted symbiont acquisition in *Stylophora pistillata*. *Symbiosis* **2026**, *99*, 261–267. [CrossRef]
31. Byler, K.A.; Carmi-Veal, M.; Fine, M.; Goulet, T.L. Multiple symbiont acquisition strategies as an adaptive mechanism in the coral *Stylophora pistillata*. *PLoS ONE* **2013**, *8*, e59596. [CrossRef] [PubMed]
32. Savary, R.; Barshis, D.J.; Voolstra, C.R.; Cárdenas, A.; Evensen, N.R.; Banc-Prandi, G.; Fine, M.; Meibom, A. Fast and pervasive transcriptomic resilience and acclimation of extremely heat-tolerant coral holobionts from the northern Red Sea. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2023298118. [CrossRef] [PubMed]
33. Louis, Y.D.; Montalbetti, E.; Isa, V.; Maggioni, D.; Pravettoni, L.; Pollutri, R.; Gobbato, J.; Montano, S.; Orlandi, I.; Vai, M.; et al. A multi-molecular biomarker assessment of thermal preconditioning in two scleractinian coral species. *Sci. Rep.* **2025**, *15*, 34785. [CrossRef] [PubMed]
34. Guillard, R.R.L.; Ryther, J.H. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* **1962**, *8*, 229–239. [CrossRef] [PubMed]
35. Hume, B.C.C.; Ziegler, M.; Poulain, J.; Pochon, X.; Romac, S.; Boissin, E.; de Vargas, C.; Planes, S.; Wincker, P.; Voolstra, C.R. An improved primer set and amplification protocol with increased specificity and sensitivity targeting the *Symbiodinium* ITS2 region. *PeerJ* **2018**, *6*, e4816. [CrossRef] [PubMed]
36. Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **1994**, *3*, 294–299. [PubMed]
37. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef] [PubMed]
38. Martino, A.; Montero, D.; Roo, J.; Holt, W.V.; Lavorano, S.; Narizzano, R.; Otero-Ferrer, F. Live microalgae-based diets as enrichment to improve the nutritional profile of the calanoid copepod *Acartia tonsa* (Dana, 1849) nauplii. *Aquac. Res.* **2023**, *2023*, 6622009. [CrossRef]
39. AOAC International. *Official Methods of Analysis of AOAC International*, 21st ed.; AOAC International: Rockville, MD, USA, 2019.
40. Folch, J.; Lees, M.; Sloane Stanley, G.H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]
41. Christie, W.W.; Han, X. *Lipid Analysis: Isolation, Separation, Identification and Lipidomic Analysis*, 4th ed.; Oily Press: Bridgwater, UK, 2010. [CrossRef]
42. Izquierdo, M.S.; Watanabe, T.; Takeuchi, T.; Arakawa, T.; Kitajima, C. Optimum EFA levels in Artemia to meet the EFA requirements of red sea bream (*Pagrus major*). In *The Current Status of Fish Nutrition in Aquaculture*; Takeda, M., Watanabe, T., Eds.; Tokyo University of Fisheries: Tokyo, Japan, 1990; pp. 221–232.
43. Yang, S.Y.; Keshavmurthy, S.; Obura, D.; Sheppard, C.R.C.; Visram, S.; Chen, C.A. Diversity and distribution of *Symbiodinium* associated with seven common coral species in the Chagos Archipelago, Central Indian Ocean. *PLoS ONE* **2012**, *7*, e35836. [CrossRef] [PubMed]
44. Tsirigoti, A.; Tzovenis, I.; Koutsaviti, A.; Economou-Amilli, A.; Ioannou, E.; Melkonian, M. Biofilm cultivation of marine dinoflagellates under different temperatures and nitrogen regimes enhances DHA productivity. *J. Appl. Phycol.* **2020**, *32*, 865–880. [CrossRef]
45. Mansour, M.P.; Volkman, J.K.; Jackson, A.E.; Blackburn, S.I. The fatty acid and sterol composition of five marine dinoflagellates. *J. Phycol.* **1999**, *35*, 710–720. [CrossRef]
46. Kihika, J.K.; Wood, S.A.; Rhodes, L.; Smith, K.F.; Miller, M.R.; Pochon, X.; Thompson, L.; Butler, J.; Schattschneider, J.; Oakley, C.; et al. Cryopreservation of six Symbiodiniaceae genera and assessment of fatty acid profiles in response to increased salinity treatments. *Sci. Rep.* **2022**, *12*, 12408. [CrossRef] [PubMed]
47. Guschina, I.A.; Harwood, J.L. Lipids and lipid metabolism in eukaryotic algae. *Prog. Lipid Res.* **2006**, *45*, 160–186. [CrossRef] [PubMed]

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