Effects of Nitrate Enrichment on Respiration, Photosynthesis, and Fatty Acid Composition of Reef Coral *Pocillopora damicornis* Larvae

Cheng-Yue Liu[1,2,3,4,5], Fang Zhang[1,4,5,6], You-Fang Sun[1,6], Xiao-Lei Yu[1,6] and Hui Huang[1,2,3,4,5]*

[1] Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China, [2] Guangdong Provincial Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China, [3] Innovation Academy of South China Sea Ecology and Environmental Engineering, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China, [4] Hainan Tropical Marine Biological Research Station, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Sanya, China, [5] Hainan Key Laboratory of Tropical Marine Biotechnology, Sanya, China, [6] University of Chinese Academy of Sciences, Beijing, China

In the foreseeable future, coastal coral reef ecosystems are likely to face further increases in eutrophication. Lipids and fatty acids (FAs), as important components of corals, are becoming a hot topic to study the effects of eutrophication on corals. This study investigated the effects of nitrate (NO_3^-) enrichment (0, 5, 10, 20, and 40 μM) on the respiration, photosynthesis, and FA compositions of *Pocillopora damicornis* larvae. Our results showed that saturated FAs (SFAs) were the most abundant in *P. damicornis* larvae over all treatment groups, followed by polyunsaturated FAs (PUFAs). The unsaturated-to-SFA ratio (U/S) and unsaturation index (UI) reduced at low nitrate concentrations (<10 μM), since the level of SFAs (mainly 16:0 and 18:0) increased whereas PUFAs (mainly 18:3n3, 20:3n6, and 22:6n3) decreased. Consequently, the biomembranes of the larvae may have become more rigid and viscous, which slowed excessive nitrate entry. Moreover, significantly enhanced photosynthetic functions of zooxanthellae in larvae were found in the N5 group (5 μM). However, the opposite FA patterns were observed in *P. damicornis* larvae at higher nitrate concentrations (>20 μM). The UI and U/S levels were elevated due to the increased PUFAs levels and decreased SFA levels at higher nitrate concentrations. Compared with the N5 and N10 groups, the fluidity of the biomembrane of the larvae did not continue to decrease but instead increased at higher nitrate concentrations, indicating that the biomembrane restructuring in the larvae may have become ineffective. Moreover, respiration increased and the consumption of numerous lipids led to a significant decrease in TFAs. These could adversely affect the dispersal, settlement, and development of larvae. Overall, *P. damicornis* larvae can adapt to low levels of nitrate (<10 μM) due to biomembrane restructuring through changes in FA composition. However, negative effects occur in larvae when nitrate exceeds 20 μM.

Keywords: *Pocillopora damicornis* larvae, fatty acid composition, nitrate enrichment, biomembrane restructuring, respiration, photosynthesis

INTRODUCTION

Reef ecosystems are among the world's most productive and biodiverse marine ecosystems (Hughes et al., 2018; Kubicek et al., 2019). Scleractinian corals serve as the framework for these ecosystems (Zaneveld et al., 2016). Coral reef waters usually contain low levels of inorganic nutrients (Renegar and Riegl, 2005). However, increased human population densities, the use of chemical fertilizers in agriculture, and domestic sewage discharge could significantly increase the input of nutrients into coastal zones (D'Angelo and Wiedenmann, 2014; Serrano et al., 2018). For the foreseeable future, coastal coral reef ecosystems are likely to face further increases in eutrophication (Humanes et al., 2016). The effect of nutrients on corals and their algal symbionts is still subject to debate. Some researchers have suggested that elevated nutrient levels can significantly decrease the growth and calcification of coral (Ferrierpagès et al., 2000; Renegar and Riegl, 2005). However, positive responses in coral to increased nutrient availability have also been found, including reduced thermal stress, zooxanthellae supplementation, and moderate bleaching (Atkinson et al., 1995; Bender-Champ et al., 2017).

Lipids and fatty acids (FAs) are the primary constituents of the cells and subcellular organelle membranes in marine organisms. They are vital structural components of biomembranes that adapt to variations in environmental conditions by changing their FA composition (Sinensky, 1974; Hazel, 1979; Wijekoon, 2011; Bennett et al., 2018). This process, called homeoviscous adaptation, can affect membrane-associated physical attributes and biological functions such as fluidity, phase behavior, thickness, permeability, and related enzymes (Yeagle, 1989; Ernst et al., 2016; Bennett et al., 2018). Lipids represent a major component of the coral composition (10–40% of dry biomass in adult corals and 34–85% of dry biomass in larvae) (Bhojoo et al., 2017; Conlan et al., 2017). Although most endogenous lipids and FAs in coral are provided by CO_2 fixation of zooxanthellae, corals also modify these lipids and FAs according to their own needs for development, reproduction, and adaptation to environmental change. Lipids and FAs are becoming a hot topic to study the effects of environmental changes on corals.

Successful dispersal, settlement, and development of larvae are critical for the maintenance of coral populations (Figueiredo et al., 2012; Jiang et al., 2019). Many physiological characteristics of coral larvae (e.g., immature cellular defenses and smaller biomass) may render them more susceptible than adult corals when exposed to the same stressors (Jiang et al., 2017; Serrano et al., 2018). However, there are few studies on the effects of nutrient enrichment on the early life stages of coral. The limited data available suggest that nitrate enrichment significantly affects performance (such as survival, growth, and settlement) (Ward and Harrison, 2000; Harrison and Ward, 2001; Lam et al., 2015; Humanes et al., 2016) and metabolism (Serrano et al., 2018). To our knowledge, no studies have yet investigated the effects of nitrate enrichment on lipids and FA composition of coral larvae.

Sanya (Hainan Province, China) is a typical tourist city, and the coastal region around it suffers from eutrophication, especially in terms of nitrates. Indeed, the nutrient levels have become significantly elevated with the rapid development of tourism and increasing population (Jing et al., 2017). Moreover, *Pocillopora damicornis* is widely distributed in this region and functions as a major hermatypic coral on reef flats. The present study assessed the respiration, photosynthesis, and FA composition of *P. damicornis* larvae with different nitrate concentrations and explored the tolerance and acclimation mechanism of *P. damicornis* larvae to nitrate enrichment from the perspective of biomembrane restructuring. These findings could provide a theoretical basis to evaluate and predict the recruitment and community reassembly of *P. damicornis* in coastal regions, especially in eutrophic areas.

MATERIALS AND METHODS

Coral Sampling and Larvae Collection

On 18 August 2018, 10 adult colonies of *P. damicornis* were collected from Luhuitou Fringing Reef (N18°12.7', E109°28.5') at depths of 2–3 m. The colonies were transported to the Tropical Marine Biological Research Station and acclimated for 24 h in fiberglass cylindrical tanks, then placed individually into 18 L flow-through tanks at ambient temperature (28.7 ± 0.5°C) under partially shaded conditions (noon irradiance, ∼300 μmol photons^{-2}s^{-1}). The outflow of each tank was passed through a cup fitted with a 180-μm net on the bottom to trap larvae. Larvae released from these colonies were collected at 07:00 on 19 August 2018 and then pooled.

Experimental Design

Laboratory experiments were performed to study the effects of nitrate enrichment on *P. damicornis* larvae. Five different treatments were established: a control group (N0) and the N5, N10, N20, and N40 groups (nitrate concentrations for each treatment are shown in **Table 1**). The *P. damicornis* larvae were stocked at a density of 300 per plastic tank (500 mL; 10.0 cm height × 10.0 cm diameter). The experiment was run for 5 days, and each treatment had three replicates. The larvae in the control group were treated with 0.5-μm-filtered seawater, and the other four nitrate-enrichment treatments involved seawater with different KNO_3 levels. The temperature was maintained at 29 ± 0.5°C, and a series of full-spectrum fluorescent bulbs (Giesemann, Nettetal, Germany) was used for irradiance (∼300 μmol photons^{-2}s^{-1}, photoperiod 12:12).

TABLE 1 | Nitrate concentration of each treatment.

Treatment	The concentration of NO_3^-/μM)
Control	2.5 ± 0.53
N5	6.8 ± 0.79
N10	11.1 ± 1.1
N20	18.5 ± 1.84
N40	36.4 ± 1.9

Values are mean ± SD.
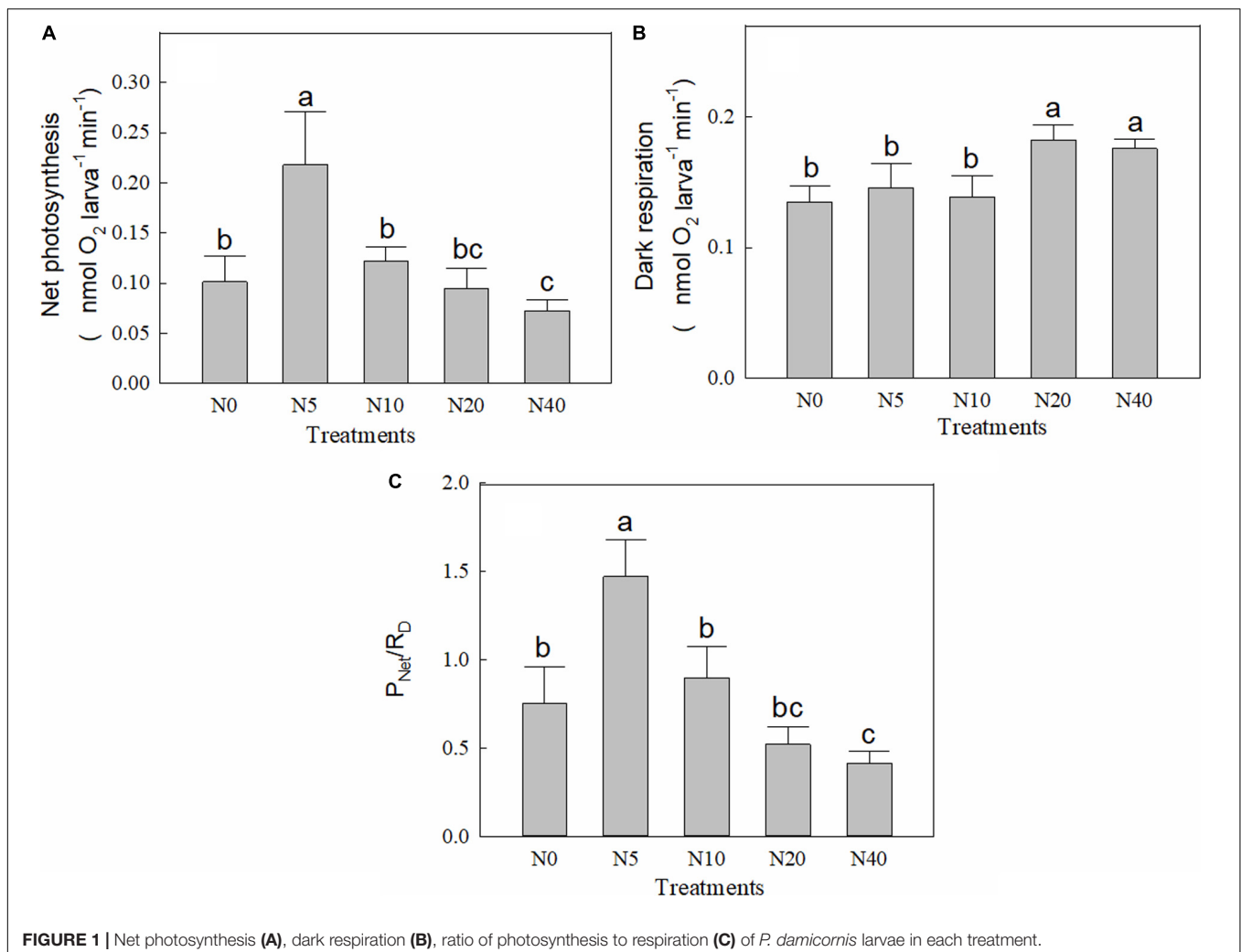
Respiration and Photosynthesis

Dark respiration (R_D) and net photosynthesis (P_{Net}) of the larvae were measured after the fifth day of the experiment. A miniature stir bar (3 × 5 mm) and 20 larvae randomly sampled from each tank were transferred into a 2-mL glass vial with an oxygen optical sensing patch on the inside wall. The filtered seawater in the vial was from the corresponding experimental tank. After turning on the magnetic stirrer (300 r/min) and dissolved oxygen meter (OXY-4 mini; Presens, Regensburg, Germany), we continuously recorded the oxygen concentration at 10-s intervals over 10 min under the same illumination as the experiment. Then, P_{Net} was calculated using the least squares linear regression of the oxygen concentration plotted against time and expressed as nanomoles of oxygen per min per larvae. R_D was measured after 2 h dark adaptation. Except that the measurement of R_D was taken with no light, the details and methods were the same as those for the P_{Net} measurements. Two additional vials containing only filtered seawater were run as the blank control for each treatment, and the results showed that the background respiration was negligible. The ratios of P_{Net} to R_D (P_{Net}/R_D) were calculated to assess autotrophic capacity (Muscatine et al., 1981). A P_{Net}/R_D ratio > 1 indicated that the net organic carbon fixed by photosynthesis of symbiotic zooxanthellae was enough to sustain the consumption of respiration. Otherwise, it implied that the endogenous reserves of the larvae were consumed.

Lipid Extraction and FA Analysis

Approximately 500 larvae (0.1 g) were collected from each group and analyzed ($n = 3$). The samples was homogenized, and each lipid fraction was extracted using chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene as an antioxidant, as previously described by Liu et al. (2019). The chloroform layer was separated from the methanol layer and dried to a constant weight under a stream of nitrogen to obtain lipids. FA methyl esters (FAMEs) were obtained by esterification with 2 mL methyl esterification reagent (hydrochloric acid/methanol, 1:5, v/v) at 90°C for 3 h, as described by Liu et al. (2018). The upper phase was dried under nitrogen and resuspended in hexane.

Fatty acid methyl esters were quantified by injecting 1 μL of sample into a gas chromatograph (GC-2010 Plus; Shimadzu,

FIGURE 1 | Net photosynthesis **(A)**, dark respiration **(B)**, ratio of photosynthesis to respiration **(C)** of *P. damicornis* larvae in each treatment.

Kyoto, Japan) equipped with a flame-ionization detector (GC-2010; Shimadzu) and an RTX-WAX fused-silica capillary column (length, 30 m; internal diameter, 0.25 mm; thickness, 0.25 μm; Phenomenex, Torrance, CA, United States). The gradient temperature program was set as follows: (i) initial temperature of 60°C for 1.0 min; (ii) increase at a rate of $10°\text{C}\,\text{min}^{-1}$ to 190°C, (iii) increase at $2.0°\text{C}\,\text{min}^{-1}$ to 260°C; (iv) hold at 260°C for 0.6 min. FAME identification and quantification were performed by comparing the retention times (identification) and peak areas (quantification) with 37-FAME Mix calibration solution (Supelco, Bellefonte, PA, United States).

Statistical Analyses

SAS statistical software version 9.4 (SAS Institute, Cary, NC, United States) was used for statistical analyses. The distribution of data was evaluated for normality with the Kolmogorov–Smirnov test ($P > 0.05$). The Levene's test ($P > 0.05$) was used to assess the homogeneity of variance. All data were then evaluated by one-way analysis of variance, followed by the Student–Newman–Keuls multiple-comparisons test to identify significant differences ($P < 0.05$) between the means of the different treatment groups. Moreover, principal component analysis (PCA) was used to display significant differences in FA profiles among the five treatment groups.

To facilitate comparison of the FA compositions, we calculated the unsaturation index (UI) and the unsaturated-to-saturated FA ratio (U/S) as reported by Wallaert and Babin (1994) and Snyder and Hennessey (2003). The UI and U/S algorithms were as follows:

$$UI = \Sigma(\%\text{monoenes} + 2 \times \%\text{dienes} + 3 \times \%\text{trienes} \ldots)/100 \tag{1}$$

$$U/S = \Sigma(\%\text{UFA})/\Sigma(\%\text{SFA}) \tag{2}$$

where monoenes, dienes, trienes... are FAs containing 1, 2, 3... double bonds, respectively; %: weight percentage; UFA: unsaturated FAs; SFA: saturated FAs.

RESULTS

Respiration and Photosynthesis of *P. damicornis* Larvae With Nitrate Enrichment

The P_{Net} of *P. damicornis* larvae showed an initial increase and then a decrease with increasing nitrate concentration (**Figure 1A**). The highest P_{Net} (0.2180 nmol O_2 larvae^{-1} min^{-1}) was observed in the N5 group, which was more than double that of the control group (N0, 0.1012 nmol O_2 larvae^{-1} min^{-1}). Subsequently, P_{Net} decreased with increasing nitrate concentration, and the lowest P_{Net} was observed in N40 (0.0726 nmol O_2 larvae^{-1} min^{-1}). Although the R_D showed no change ($p > 0.05$) at nitrate concentrations ≤ 10 μM (N0, N5, and N10 groups), it significantly increased ($p < 0.05$) in the

N20 and N40 groups (**Figure 1B**). The ratio of P_{Net}/R_D ranged from 0.4151 to 1.4724 (**Figure 1C**). The P_{Net}/R_D showed similar changes to those of P_{Net} and, except for N5, P_{Net}/R_D of the treatment groups were less than 1. The lowest P_{Net}/R_D was in the N40 group (0.4151).

Total Fatty Acids (TFAs) of *P. damicornis* Larvae

The TFA levels present in the *P. damicornis* larvae after each treatment are shown in **Table 2**. When the nitrate concentration was less than 10 μM (N0, N5, and N10 groups), the TFA levels in *P. damicornis* larvae were not significantly different ($p > 0.05$). When the nitrate concentration was further elevated, the TFAs significantly decreased ($p < 0.05$). The lowest TFA levels (45.87 μg/g) were observed when the nitrate concentration was 40 μM (N40 group), and were less than half those in the control group.

FA Compositions of *P. damicornis* Larvae

Twenty-three FA species were identified among the *P. damicornis* larvae, including nine SFAs, eight monounsaturated FAs (MUFAs), and nine polyunsaturated FAs (PUFAs). Seven major FAs were recurrently found in *P. damicornis* larvae, including palmitic acid (16:0), stearic acid (18:0), heneicosanoic acid (21:0), oleic acid (18:1n9), palmitoleic acid (16:1n7), EPA (20:5n3), and DHA (22:6n3).

The *P. damicornis* larvae had the highest abundance of SFAs (more than 60%), followed by PUFAs (approximately 30%), and the lowest abundance of MUFAs (only 10%). The predominant SFA, MUFA, and PUFA were 16:0, 16:1n7, and 22:6n3, respectively. Moreover, the UI ranged between 1.35 and 1.07, and the U/S ratio ranged between 0.65 and 0.51. The maximum and minimum UI and U/S levels occurred in the N5 and N0 groups, respectively.

FA Composition Change in *P. damicornis* Larvae With Nitrate Enrichment

The FA profiles of the *P. damicornis* larvae with different nitrate enrichments are shown in **Table 3** and **Figure 2**. To better visualize the FA changes in the *P. damicornis* larvae with different nitrate concentrations, six line charts for UI, U/S, SFA, MUFA, and PUFA are presented in **Figure 3**. When the nitrate concentration was less than 10 μM, the SFA levels in the *P. damicornis* larvae were significantly elevated, primarily due to increased 16:0 and 18:0 levels and decreased PUFA concentrations (18:2n6, 18:3n3, 20:3n6, and 22:6n3), with corresponding reductions in UI and U/S.

In contrast, the SFA levels of larvae decreased and the UFAs increased in the N20 group, leading to elevated UI and U/S. Compared with the N20 group, no significant difference ($p > 0.05$) was observed in the FA profiles of the *P. damicornis* larvae in the N40 group. Although the levels of some monoenes altered significantly throughout the

TABLE 2 | Total fatty acids of *Pocillopora damicornis* larvae with different nitrate concentrations (μg/g).

Nitrate	0	5	10	20	40	*P*-value
TFA	97.08 ± 1.09[a]	90.77 ± 0.58[a]	88.8 ± 0.22[a]	57.37 ± 0.26[b]	45.87 ± 0.45[c]	<0.0001

Values are mean ± SD, significant differences (P < 0.05) each row are indicated by different lower-case letters. TFA, total fatty acids.

TABLE 3 | Fatty acid compositions (% total fatty acids) of *P. damicornis* larvae with different nitrate concentrations.

Nitrate	0	5	10	20	40	*P*-value
Saturated fatty acids						
14:0	4.18 ± 0.15[a]	3.96 ± 0.04[ab]	3.86 ± 0.02[b]	3.91 ± 0.02[b]	4.02 ± 0.15[ab]	0.0204
15:0	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.09 ± 0.01	0.08 ± 0.02	0.0622
16:0	32.69 ± 0.33[b]	33.99 ± 0.36[a]	34.26 ± 0.31[a]	32.66 ± 0.12[b]	32.63 ± 0.1[b]	< 0.0001
17:0	0.10 ± 0.01[b]	0.11 ± 0.01[b]	0.16 ± 0.02[a]	0.12 ± 0.01[b]	0.11 ± 0.01[b]	0.0016
18:0	14.23 ± 0.71[c]	17.63 ± 0.57[a]	20.3 ± 0.09[a]	17.16 ± 0.22[b]	16.94 ± 0.14[b]	< 0.0001
20:0	0.86 ± 0.02[a]	0.74 ± 0.02[b]	0.83 ± 0.07[a]	0.80 ± 0.01[ab]	0.79 ± 0.01[ab]	0.0116
21:0	6.95 ± 0.43[a]	5.77 ± 0.17[c]	5.51 ± 0.22[c]	6.43 ± 0.03[b]	6.44 ± 0.06[b]	0.0001
22:0	0.16 ± 0.01	0.14 ± 0.01	0.16 ± 0.02	0.15 ± 0.00	0.15 ± 0.00	0.2055
23:0	1.45 ± 0.21	1.51 ± 0.24	1.28 ± 0.06	1.38 ± 0.08	1.39 ± 0.02	0.4752
ΣSFA	60.69 ± 0.9[d]	63.92 ± 0.18[b]	66.43 ± 0.37[a]	62.68 ± 0.1[c]	62.54 ± 0.11[c]	< 0.0001
Monounsaturated fatty acids						
14:1n5	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.2237
15:1n5	0.77 ± 0.03	0.88 ± 0.06	0.80 ± 0.11	0.72 ± 0.01	0.77 ± 0.01	0.0008
16:1n7	4.29 ± 0.16	4.33 ± 0.22	4.03 ± 0.00	4.09 ± 0.01	4.13 ± 0.05	0.0049
17:1n7	0.06 ± 0.01[b]	0.27 ± 0.37[ab]	0.64 ± 0.00[a]	0.56 ± 0.00[a]	0.46 ± 0.07[a]	0.0115
18:1n9	4.40 ± 0.40[a]	3.50 ± 0.13[b]	3.59 ± 0.11[b]	3.78 ± 0.01[b]	3.75 ± 0.02[b]	0.0016
20:1n9	1.01 ± 0.17[a]	0.78 ± 0.02[b]	0.79 ± 0.04[b]	0.82 ± 0.00[b]	0.81 ± 0.01[b]	0.0239
22:1n9	0.07 ± 0.01[ab]	0.08 ± 0.02[a]	0.05 ± 0.01[ab]	0.06 ± 0.00[ab]	0.05 ± 0.01[b]	0.0335
24:1n9	0.13 ± 0.03	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.00	0.09 ± 0.04	0.2075
ΣMUFA	10.79 ± 0.67	10.01 ± 0.64	10.07 ± 0.07	10.22 ± 0.02	10.1 ± 0.12	0.2248
Polyunsaturated fatty acids						
18:2n6	1.77 ± 0.11[a]	1.58 ± 0.14[b]	1.47 ± 0.00[b]	1.58 ± 0.00[b]	1.56 ± 0.02[b]	0.0112
18:3n3	5.93 ± 0.38[a]	5.43 ± 0.15[bc]	5.2 ± 0.04[c]	5.7 ± 0.04[ab]	5.7 ± 0.08[ab]	0.0063
18:3n6	1.64 ± 0.02	1.65 ± 0.08	1.52 ± 0.07	1.63 ± 0.01	1.64 ± 0.01	0.0399
20:2n6	0.92 ± 0.23	0.86 ± 0.05	0.76 ± 0.11	0.89 ± 0.01	0.91 ± 0.00	0.4375
20:3n6	3.18 ± 0.18[a]	3.11 ± 0.04[ab]	2.87 ± 0.03[b]	2.95 ± 0.09[b]	2.92 ± 0.11[b]	0.018
20:4n6	0.51 ± 0.11	0.47 ± 0.05	0.47 ± 0.00	0.50 ± 0.00	0.50 ± 0.01	0.8297
20:5n3	2.36 ± 0.27	2.39 ± 0.13	2.12 ± 0.09	2.38 ± 0.02	2.42 ± 0.02	0.134
22:2n6	0.14 ± 0.02[b]	0.23 ± 0.04[b]	0.74 ± 0.19[a]	0.61 ± 0.02[a]	0.61 ± 0.02[a]	< 0.0001
22:6n3	12.07 ± 0.45[a]	10.38 ± 0.27[c]	8.35 ± 0.36[d]	10.87 ± 0.03[bc]	11.09 ± 0.06[b]	< 0.0001
ΣPUFA	28.52 ± 0.57[a]	26.11 ± 0.53[c]	23.5 ± 0.44[d]	27.1 ± 0.11[b]	27.35 ± 0.01[b]	< 0.0001
UI	1.35 ± 0.03[a]	1.22 ± 0.02[c]	1.07 ± 0.03[d]	1.26 ± 0.00[b]	1.28 ± 0.00[b]	< 0.0001
U/S	0.65 ± 0.02[a]	0.57 ± 0.00[c]	0.51 ± 0.00[d]	0.60 ± 0.00[b]	0.60 ± 0.00[b]	< 0.0001

Values are mean ± SD, significant differences (P < 0.05) each row are indicated by different lower-case letters. MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; UI, unsaturated index; U/S, the ratio of unsaturated and saturated fatty acid.
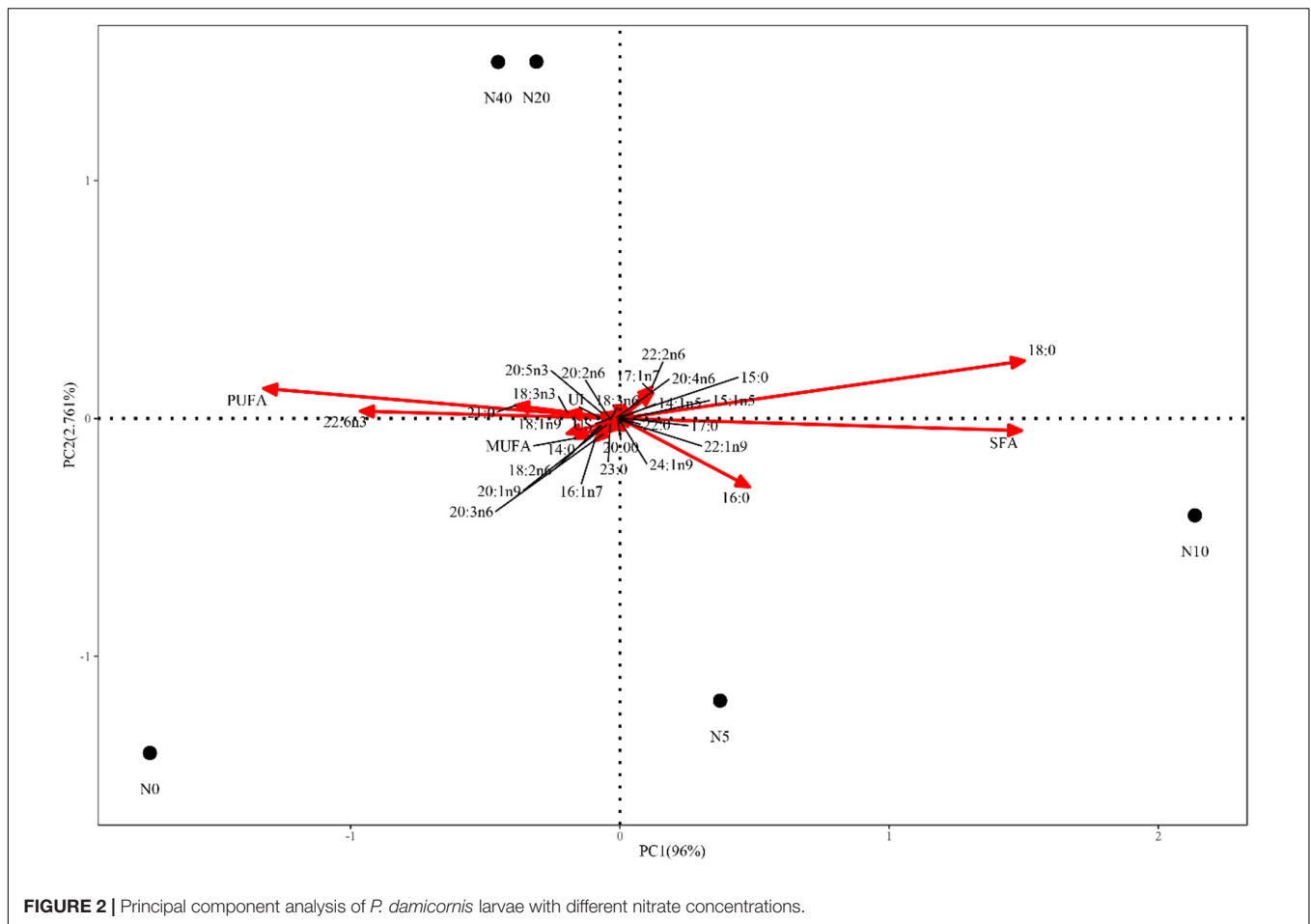
experiment (major change in 18:1n9), the total MUFA levels of the larvae did not change.

DISCUSSION

Respiration and Photosynthesis of *P. damicornis* Larvae With Nitrate Enrichment

Among all the groups, N5 had the highest P_{Net} and P_{Net}/R_D, which implied that low nitrate enrichment could promote photosynthesis in coral larval zooxanthellae. Marubini and Davies (1996) reported that low nitrate enrichment (>5 μM) enhances the photosynthesis of coral zooxanthellae by increasing zooxanthellae size and chlorophyll concentrations. Atkinson et al. (1995) also demonstrated that coral living in aquaria can thrive in relatively high-nutrient water (nitrate: about 5 μM). Nonetheless, coral species-specific responses to nitrate enrichment have been found in previous studies (Ferrier-Pagès et al., 2001; D'Angelo and Wiedenmann, 2014; Serrano et al., 2018). Contrary to our findings, Ferrier-Pagès et al. (2001) found that nitrate enrichment had no effect on *Stylophora pistillata*.
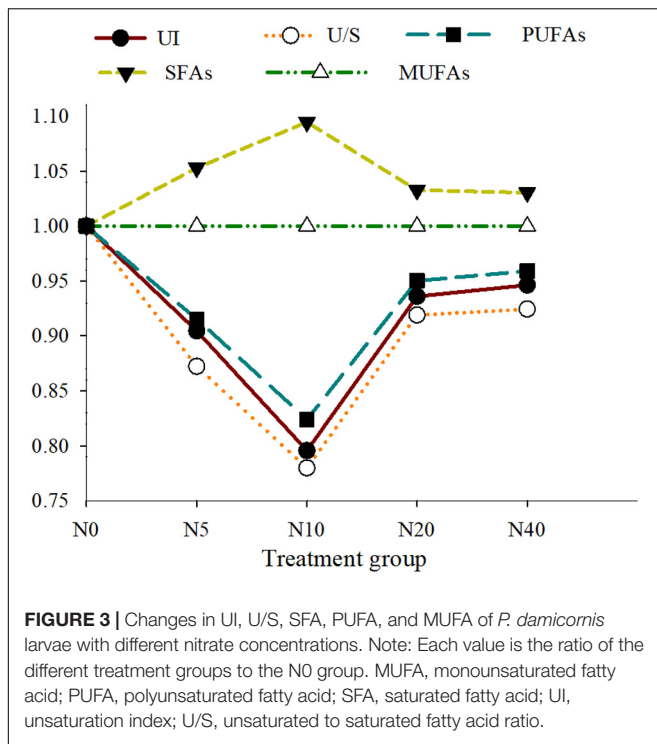
FIGURE 2 | Principal component analysis of *P. damicornis* larvae with different nitrate concentrations.

In this study, relatively higher nitrate concentrations produced P_{Net} decreases and R_D increases, resulting in decreased P_{Net}/R_D. This indicated that high levels of nitrate (>20 μM) would have a negative impact on *P. damicornis* larvae. Serrano et al. (2018) found that nitrate enrichment (12 μM) could significantly increase respiration in *Porites astreoides* larvae. Since R_D represents the metabolic rate of coral larvae, we hypothesized that *P. damicornis* larvae increase respiration in order to provide enough energy to ameliorate environmental stress. Nordemar et al. (2003) documented that the primary production of *Porites cylindrica* declined after 2 weeks of nitrate enrichment (15 μM), while zooxanthellae density and chlorophyll concentrations (photosynthesis) remained unaltered. Alternatively, increased respiration rates in nutrient-enriched *P. damicornis* larvae may affect their stress response to potential photo-physiological damage to their algal symbionts (D'Angelo and Wiedenmann, 2014).

FA Compositions of *P. damicornis* Larvae
In this study, the SFA level (mainly 16:0 and 18:0) was the highest (over 60%) for FAs in the *P. damicornis* larvae. The FA patterns of *P. damicornis* larvae were similar to those of adults of this species (previous unpublished research) and to those of *Goniastrea retiformis* (Figueiredo et al., 2012),

Acropora millepora, *Acropora tenuis*, and *Montipora digitata* eggs (Arai et al., 1993). Both Papina et al. (2003) and Chen et al. (2015) found that palmitic acid (16:0) acted as a trophic marker of zooxanthellae and was abundant in coral larvae, especially those that inherited algal symbionts maternally from oocytes. *P. damicornis* release larvae that carry symbiotic micro-algae, which may explain the higher SFAs observed in our study. Moreover, compared to PUFAs, SFAs have a more stable structure and are commonly used for bio-energy storage. Many investigators have reported that SFAs (such as 16:0 and 18:0) are the preferred sources of metabolic energy in marine organisms (Arai et al., 1993; Figueiredo et al., 2012; Wijekoon, 2012). Consequently, *P. damicornis* larvae need to store sufficient SFAs to have a capacity to delay metamorphosis until suitable settlement cues appear.

Likewise, a high abundance of several PUFAs (such as 18:3n3, 20:3n6, 20:5n3, and 22:6n3) was found. This may be due to the fact that symbiotic zooxanthellae contain high PUFA levels (Zhukova and Titlyanov, 2003) and symbionts transfer PUFAs to host tissue (Figueiredo et al., 2012), after which they are incorporated into their larvae. Although the specific functions of PUFAs for coral remain poorly studied, PUFAs, especially the n-3 and n-6 PUFAs, have been confirmed as critical to the growth, survival, and reproduction of many organisms, including corals

FIGURE 3 | Changes in UI, U/S, SFA, PUFA, and MUFA of *P. damicornis* larvae with different nitrate concentrations. Note: Each value is the ratio of the different treatment groups to the N0 group. MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; UI, unsaturation index; U/S, unsaturated to saturated fatty acid ratio.

(Tchernov et al., 2004; Imbs, 2013; Chen et al., 2015; Ernst et al., 2016; Conlan et al., 2017).

Effect of Nitrate Enrichment on the FA Composition of *P. damicornis* Larvae

Significant changes in the FA compositions of *P. damicornis* larvae were observed under nitrate enrichment. Our results indicate that the lipids of *P. damicornis* larvae become progressively saturated (lower UI and U/S) when nitrate is less than 10 μM, which can be attributed to decreased PUFAs and a concomitant increase in SFAs. Moreover, no changes in TFA levels were found at the lower nitrate concentrations. Lipids and proteins are the primary constituents of biomembranes. Changes in FA composition could affect membrane-associated physical attributes and biological functions, such as membrane-phase behaviors, membrane thickness, and membrane permeability (Losa and Murata, 2004; Wijekoon, 2012; Ernst et al., 2016). Compared to UFAs, homologous SFAs have a higher melting point and occupy a smaller space within the membrane lipid bilayer, which enhances their rigidity and viscosity (Hazel, 1979; Yeagle, 1989; Liu et al., 2018). The more rigid and viscous the biomembrane, the more slowly harmful ions enter the organism. Thus, the present data indicated that *P. damicornis* larvae can adapt to low-level nitrate enrichment by biomembrane restructuring. While it is well established that FAs play an important role in stress resistance and that the ability of an organism to maintain appropriate membrane function integrity and cell homeostasis in the face of environmental change is intimately linked to tolerance, this

is the first time that these responses have been demonstrated in coral larvae.

Moreover, with increases in environmental stress, organisms may produce more reactive oxygen species (ROS) (Cunning and Baker, 2013; Liu et al., 2018). Irrespective of the MUFA or PUFA contents, a higher proportion of UFAs in lipids was associated with increased ROS susceptibility, whereas SFAs did not undergo lipid peroxidation (Porter, 2013; Cengiz et al., 2017). Organisms with a higher proportion of SFAs could reduce the influence of ROS (Wada et al., 1994; Crockett, 2008; Liu et al., 2019). Thus, such adaptations with respect to lipids can minimize the influence of nitrate enrichment on *P. damicornis* larvae. Similarly, with nitrate enrichment, thermal stress can significantly affect the coral FA composition. While studying symbiotic algae in thermally bleached corals, Tchernov et al. (2004) found that higher SFAs enhanced the thermal stability of the eukaryotic thylakoid membranes of coral and simultaneously reduced the susceptibility of the membrane lipids to attacks by ROS.

One interesting result was that while UFAs were lowest in the N10 group, the P_{Net} dropped compared to the N5 group. Although higher SFA can increase biomembrane rigidity and anti-oxidation, it reduces membrane-bound enzyme activity and material exchange (Oku et al., 2002; Bachok et al., 2006; Ernst et al., 2016), which may reduce the photosynthesis of zooxanthellae. Bennett et al. (2018) reported that PUFA enrichment in the phototrophic sponge *Carteriospongia foliascens* can significantly facilitate enzyme activity. Ernst et al. (2016) showed that lipids with saturated acyl chains are packed at higher densities and tend to form non-fluid gel phases, which slow substance exchange.

Different FA patterns were observed in the *P. damicornis* larvae in the N20 and N40 groups. The UI and U/S levels were elevated due to the increased PUFA levels and decreased SFA levels at higher nitrate concentrations. Although the nitrate concentration increased, compared with the N5 and N10 groups, the fluidity of the biomembrane of the larvae did not continue to decrease but instead increased at higher nitrate concentrations, indicating that the biomembrane restructuring in the larvae may have become ineffective. In parallel, the TFAs decreased significantly. This result showed that numerous lipids were consumed rapidly. When ions exceed the threshold that the larvae can tolerate (hyperosmotic stress), the larvae need to excrete the ions via active transport in order to maintain homeostasis (Losa and Murata, 2004). Thus, a large amount of SFA, as the preferred source of metabolic energy, is oxidized to provide energy for this process. This is consistent with our findings that *P. damicornis* larval R_D increased significantly at high nitrate concentrations (N20 and N40 groups). In addition, to some extent, the increase in UFAs could promote biomembrane fluidity, enzyme activity, and internal and external exchange of substances that could enhance metabolism (Brenner, 1984; Bennett et al., 2018; Liu et al., 2018). However, a higher proportion of UFAs would reduce the antioxidant capacity of larvae. Hence, numerous lipids were consumed, and the increased risk of lipid peroxidation could adversely affect the successful settlement and development of the larvae.

CONCLUSION

The present research showed that, in all treatment groups, *P. damicornis* larvae had the highest proportion of SFAs, followed by PUFAs, and the lowest proportion of MUFAs. The FA pattern of larvae could store sufficient energy to delay metamorphosis until suitable settlement cues appeared. Nitrate enrichment significantly changed the FA composition of *P. damicornis* larvae. The lipids of *P. damicornis* larvae became progressively saturated (lower UI and U/S) when the nitrate concentration was less than 10 μM, which could be attributed to decreased PUFAs and concomitant increases in SFAs. Such changes enabled the *P. damicornis* larvae to adapt to low-level nitrate enrichment (<10 μM). Moreover, the N5 group had the highest P_{Net} and P_{Net}/D_R, which implied that low nitrate enrichment could promote photosynthesis of coral larvae zooxanthellae. However, the opposite pattern for FA compositions was found with higher nitrate concentrations (>20 μM), in which the UI and U/S levels were elevated due to increased PUFAs and decreased SFAs. With high nitrate levels, biomembrane restructuring in larvae may became ineffective, increasing respiration and rapidly consuming numerous lipids, which could adversely affect the successful settlement and development of larvae.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00531/full#supplementary-material

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