Oceanologia (2016) 58, 272—278

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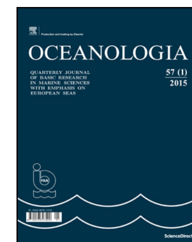

ORIGINAL RESEARCH ARTICLE

The effect of temperature and nitrogen deprivation on cell morphology and physiology of *Symbiodinium*

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Received 4 June 2015; accepted 22 April 2016
Available online 10 May 2016

KEYWORDS
Symbiodinium;
Temperature;
Nitrogen deprivation

Summary Nutrients and temperature are the major elements in maintaining stable endosymbiotic relationships. The mechanisms and response of cultured *Symbiodinium* cells in the absence of nitrogen, and at various temperatures are still unclear. The present study investigated the influence of different temperatures and nitrogen-deprivation on free-living *Symbiodinium* cultures. The physiological responses of free-living *Symbiodinium* cells cultured at different temperatures during nitrogen deprivation under a 12:12 h light:dark were measured. *Symbiodinium* cell growth was significantly lower in response to lower temperatures. Transmission electron micrographs (TEMs) revealed the formation of lipid droplets induced by nitrogen deprivation under different temperatures. The results of this study will increase our understanding of adaptive responses occurring in *Symbiodinium* under environmental stress.
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Peer review under the responsibility of Institute of Oceanology of the Polish Academy of Sciences.

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http://dx.doi.org/10.1016/j.oceano.2016.04.006
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1. Introduction

Symbiodinium sp. is a unicellular microalga that has mutualistic associations with invertebrates such as corals and anemones (Davy et al., 2012). The coral host provides nutrients and carbon dioxide to *Symbiodinium* in hospite (Muscatine and Porter, 1977; Rodrigues and Grottoli, 2007). In return, these microalgae transfer more than 90% of their photosynthetically fixed carbon to the host cytoplasm where they reside (Muscatine, 1980). This mutualistic relationship enables coral to survive and remain healthy.

In more than a decade, the phenomenon of global climate change has contributed to the decline of coral reef around the world (Wilkinson, 2008). In such case, this change might generate huge impacts to the ocean ecosystem at all levels, such as higher mean sea water level, warmer ocean, stratified ocean, increasing CO_2 level in global scale, ice cover and ocean chemistry changes (Doney et al., 2009; Duce et al., 2008; Jackson et al., 2001; Kroecker et al., 2013; Poloczanska et al., 2013). The coral bleaching is expected to happen more frequently and become more severe due to the climate change. Environmental factors, such as nutrient levels and temperatures, play an important role in maintaining the stability of mutualistic associations between cnidarians and unicellular dinoflagellates (Belda et al., 1993; Hastie et al., 1992; Hoegh-Guldberg and Smith, 1989; Steen and Muscatine, 1987). It was reported that coral bleaching appeared in the seawater of southern Taiwan due to the exposure of temperature at 30—31°C (Mayfield et al., 2013, 2011). Furthermore, when corals were exposed at 14°C under the full sunlight, the reduction in photosynthetic ability caused the coral bleaching (Saxby et al., 2003). In terms of elevated sea temperature, the stability of corals and *Symbiodinium* endosymbiotic relationship is yet to be determined by anthropogenic stress factor such as coastal water quality (Wooldridge, 2014). Previous studies show that increasing nutrient levels, like dissolved nitrogen (N), in coastal water could induce some physiological impact on coral-dinoflagellate, for example, coral bleaching and reduced symbiont density (Koop et al., 2001; Marubini and Davies, 1996; Stimson, 1991; Szmant, 2002; Wiedenmann et al., 2013). For instance, the organic carbon supply by *Symbiodinium* to hosts could be established depending on nutrients from various sources including exogenous sea water, host catabolism and host heterotrophy (Steen, 1986; Szmant-Froelich and Pilson, 1984). Nitrogen, a major nutrient, is excreted as ammonium by the host (Rahav et al., 1989). Several investigations have reported that endosymbionts could survive in nitrogen-limited environments (Jiang et al., 2014; Peng et al., 2012). Therefore, nitrogen deprivation could alter symbiont physiologies (Weng et al., 2014). It is likely that symbiotic cnidarians may maintain the endosymbiont density through regulation of nitrogenous waste (McAuley, 1987; Rees, 1989). Moreover, temperature elevation alone can damage *Symbiodinium* cells in hospite (Sammarco and Strychar, 2013). It has been reported that the nutrient uptake in symbionts differed under different temperatures due to stress susceptibility among corals hosting different symbionts (Baker et al., 2013). There have been numerous studies using *Symbiodinium* treated with either differing temperatures or nitrogen deprivation (Jiang et al., 2014; Nitschke et al., 2015; Weng et al., 2014).

Several studies have reported that nitrogen starvation or other environmental stressors can affect growth, morphology, and metabolism of microalgae (Hockin et al., 2012; Pasaribu et al., 2014). For example, reduced nitrogen concentration increases lipid production in microalgae, which is stored in lipid droplets (Li et al., 2010; Piorreck et al., 1984). Recent studies have shown that increasing the temperature variation induced lipid content accumulation in *Nannochloropsis oculata* (Converti et al., 2009). Macedo and Alegre (2001) observed that lipid content increased in *Spirulina* cultured with nitrogen and decreased temperatures. However, the synergistic effects between nitrogen source and different temperature treatment on cellular mechanisms of *Symbiodinium* are poorly known.

The present study describes the influence of different temperatures and the absence of nitrogen in the medium on cultured free-living *Symbiodinium*. The aim was to examine changes in cellular biology, including cell proliferation, lipid classes, and ultrastructure in free-living *Symbiodinium*. *Symbiodinium* cell proliferation was slower when samples were cultured at temperatures of 15°C, than at 25 and 30°C. Results showed increased formation of different lipid droplets in *Symbiodinium* when cultured in extreme temperatures (i.e. 15 and 30°C).

2. Material and methods

2.1. *Symbiodinium* culture and treatment

The free-living *Symbiodinium* sp. (clade B) used in this study was obtained from National Museum of Marine Biology and Aquarium. They were maintained in the f/2 medium in filtered seawater (FSW) at room temperature under a photosynthetically active radiation (PAR) of 40 μmol m^{-2} s^{-1} in a 12-h light/12-h dark (12L/12D) cycle. For treatment, three-batch cultures were grown in the nitrogen-deficient artificial seawater with temperatures at 15, 25 and 30°C, separately.

2.2. *Symbiodinium* clade identification

The genetic identity (18S rDNA) of the cultured *Symbiodinium* was examined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis, and it was determined as clade B. *Symbiodinium* DNA was extracted using a plant genomic DNA extraction miniprep system (VIOGENE, Taipei). Basically, *Symbiodinium* nuclear small subunit (n18S-rDNA) was amplified by PCR from 3 replicate extracts of each of the two cultures using the primers, ss5z (an equimolar mixture of the oligonucleotides 5′-GCAGTTATAATT TATTTGATGGTCACTGCTAC-3′ and 5′-GCAGTTATAGTTTATTT-GATGGTTGCTGCTAC-3′) and ss3z (5′-AGCACTGCGTCAGTCC-GAATAATTCACCGG-3′). The PCR products were digested by restriction enzymes, *Taq* I and *Sau*3A I (Promega, USA). The digestion products were separated by electrophoresis on 1.5% 0.5× TAE (Amresco, USA) agarose gels, to generate the RFLP pattern. The RFLP pattern was compared to the literature (Rowan and Powers, 1991) to identify the clade of each culture.

2.3. Cell density determination

The *Symbiodinium* cell density was counted with haemocytometer based cell counting. Cell densities were determined daily by placing an aliquot of well-mixed culture suspension on a Neubauer hemocytometer (Marienfel, Germany) under a Axioskop2 Plus microscope (Zeiss, Germany) connected to a CCD camera (Photometrics, USA).

2.4. Lipid analyses

Lipid contents of three replicates from the nitrogen-deprived culture *Symbiodinium* cells with temperatures 15, 25 and 30°C were extracted by the Bligh and Dyer procedure (Bligh and Dyer, 1959). Neutral lipids from *Symbiodinium* cells were extracted with 150 μl of chloroform/methanol (2:1, v/v). After centrifugation, the lower chloroform fraction was collected for the analysis by thin layer chromatography (TLC) (Analtech, USA) with the solvent system modified from previous reports (Fuchs et al., 2007; Oku et al., 2003).

2.5. The transmission electron microscopy and imaging analysis

To investigate the morphological variability of *Symbiodinium* cultured in different temperatures under nitrogen deprivation, *Symbiodinium* cells were collected and fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 100 mM sodium phosphate containing 5% sucrose (pH 7.3) for 2.5 h at 4°C. They were then rinsed with 100 mM sodium phosphate buffer at 4°C. Cells were then post-fixed in 1% OsO_4 in 50 mM sodium phosphate (pH 7.3) for 1 h at 4°C. The cell aliquots were then washed three times for 15 min each with the same buffer and dehydrated by a graded ethanol series (50, 70, 80, 90, 95 and 100%) before embedding in LR white resin. Thin sections (70 nm) cut by a Leica Reichert Ultracut R were collected on nickel grids, post-stained with 2.5% uranyl acetate and 0.4% lead citrate, rinsed three times with water, and the samples were viewed on a JEM-1400 transmission electron microscope (JEOL, Japan).

3. Results and discussion

3.1. Morphology of free living cultured *Symbiodinium* cells

Symbiodinium spp. can be either autotrophic or symbiotic in cnidarians cells. Free-living *Symbiodinium* cells of 5—6 μm were brown-colored and coccoid-shaped (Fig. 1A). The size of cultured free-living *Symbiodinium* cells was equivalent to those of other free-living *Symbiodinium* species and clades (Blank and Huss, 1989). Generally, the morphology of cultured free-living *Symbiodinium* cells indicated they were smaller than symbiotic cells isolated from coral (Pasaribu et al., 2014). It has been reported that the most abundant *Symbiodinium* is coccoid yellow-brown (Freudenthal, 1962), and is smaller than cultured *Symbiodinium* (Jeong et al., 2014). In cultured free-living *Symbiodinium*, the intercalary bands were located at the cell surface (Fig. 1B). The intercalary bands are known as growth bands indicating cell growth, and occur with the addition of material along plate margins in some dinoflagellates (Graham and Wilcox, 2000).

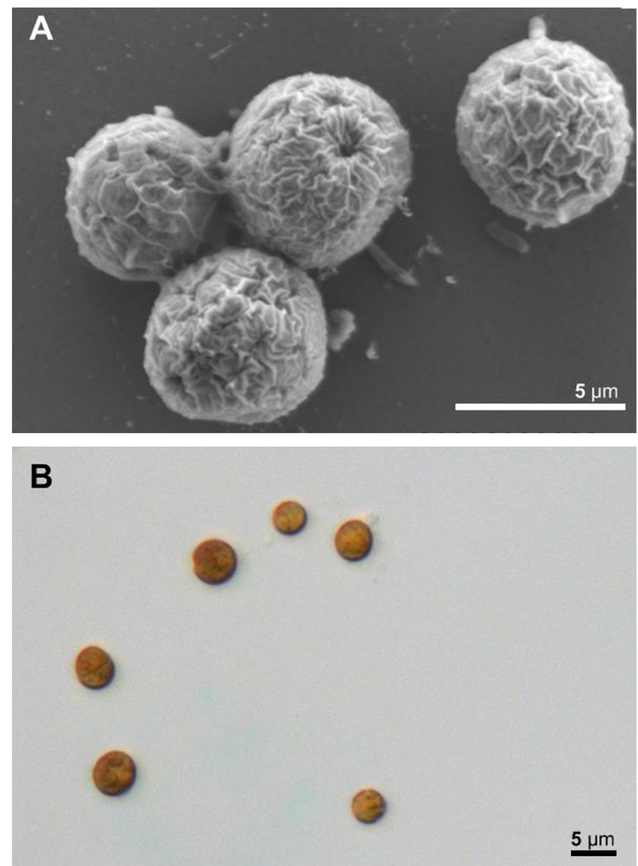

Figure 1 Microscopic examination of free-living cultured *Symbiodinium*. (A) Light microscopy of free-living cultured *Symbiodinium*. (B) Free-living cultured *Symbiodinium* observed under scanning electron microscopy.

3.2. Effect of temperature and nitrogen-deprivation on *Symbiodinium* cell growth

Temperature is an important factor for algal growth. It strongly influences cellular chemical composition, nutrient uptake, and growth rates of all algal species. After 5 days, *Symbiodinium* cells cultivated in a nitrogen-deprived medium proliferated more slowly and showed increased lipid contents, than those in a nitrogen-enriched medium (Jiang et al., 2014). To determine the most efficient way to cultivate *Symbiodinium* cells and induce lipid accumulation, free-living *Symbiodinium* cells were subjected to different temperatures under nitrogen-deprivation, and the cultures were counted using a hemocytometer. Fig. 2 shows that when cultured under nitrogen-starvation, *Symbiodinium* cells have slower growth at lower temperatures (15°C) than at normal temperatures (25°C). However, at high temperatures (30°C) growth rate was higher than at normal temperatures (25°C) (Fig. 2). As *Symbiodinium* cells cultured in nitrogen-deprived media showed inhibited growth with lower temperatures, higher temperatures were considered appropriate to achieve high cell concentrations. Different temperatures also affected phosphorus uptake in microalgae, with phosphorus

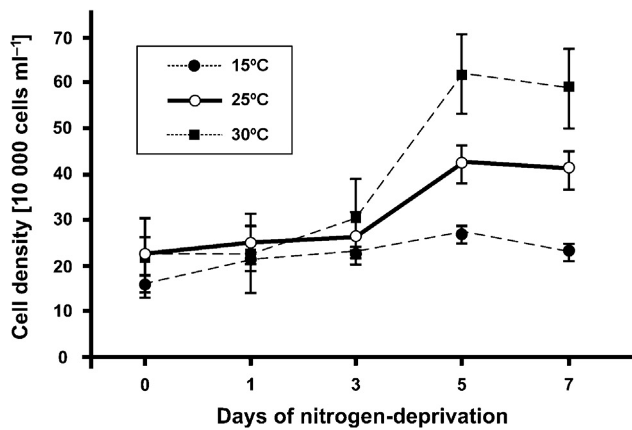

Figure 2 Growth curves of *Symbiodinium* cells cultivated at different temperatures under nitrogen deprivation. Temperature treatments (15, 25, and 30°C) were applied to free-living *Symbiodinium* sp. The data represents means ± SD (n = 3).

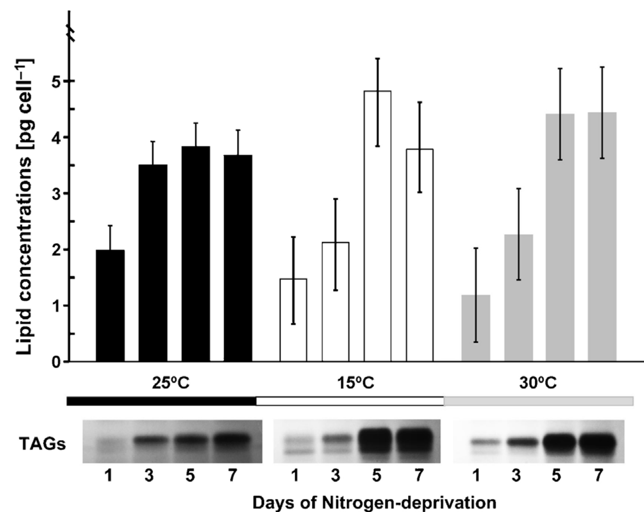

Figure 3 Thin layer chromatography (TLC) analysis of lipids extracted from *Symbiodinium* cells cultivated at different temperatures under nitrogen deprivation. The lipid comparison of free-living cultured *Symbiodinium* between three different temperatures under nitrogen deprivation.

accumulation increasing with higher temperatures compared to lower temperatures (Powell et al., 2008). Several microalgal species can grow in extreme environments, such as high temperatures of 30—35°C (Converti et al., 2009; Oliveira et al., 1999), however, some species do not grow efficiently in temperatures above 30°C and these temperatures are considered as heat stress (Bajguz, 2009). *Symbiodinium* cells had the best growth rates at normal temperatures (25°C) with nitrogen supply (Pasaribu et al., 2015); however, results from the present study indicated that *Symbiodinium* cells grew well in temperatures above 30°C in nitrogen-free media. Other studies have reported similar results that the optimum growth of *Symbiodinium* could exceed the thermal break-point (>32°C) (Kinzie et al., 2001; Strychar et al., 2005). Furthermore, the response to the temperature changes was an adaptive physiological adjustment of *Symbiodinium* (Sammarco and Strychar, 2013). It is feasible that *Symbiodinium* endosymbiont were present in nutrient-limited environment with respect to nitrogen source (Dubinsky and Berman-Frank, 2001; Wooldridge, 2010), therefore, the stability of coral-dinoflagellate was particularly supported by interaction of temperature and nutrient.

3.3. Temperature and nitrogen-deprivation induces neutral lipid accumulation in *Symbiodinium* cells

Nitrogen deprivation is a nutrient limitation that disrupts algal cell metabolism. Several studies reported that microalgae cultivation under environmental stress caused slow growth and lipid accumulation (Hu and Gao, 2005; Yeesang and Cheirsilp, 2011). Cultured free-living *Symbiodinium* cells were treated with low and high temperatures in a nitrogen-free medium for 7 days. The change in lipid content during the treatments was analyzed using thin-layer chromatography (TLC) (Fig. 3). There were no apparent differences between the temperatures during the experiment, and all temperature treatments induced neutral lipid (triacylglyceride [TAG]) accumulation on day 5. Previous studies demonstrated that nitrogen deprivation increased lipid content and lipid droplet accumulation in free-living *Symbiodinium* cells (Jiang et al., 2014). These findings were similar to those of the studies involving temperature and nutrient-starved green algae, *Spirulina* sp. and *N. oculata* (Converti et al., 2009; Macedo and Alegre, 2001).

3.4. Ultrastructure of lipid droplets in *Symbiodinium* cells

The main cause of coral bleaching is extreme temperatures and if the extreme temperature persists for a long period, corals begin to die. Sammarco and Strychar (2013) concluded that during exposure to the high temperatures for soft coral for 2 days, the *Symbiodinium* cells were apoptotic, but the hosts adapted to the high temperature. High water temperatures can affect the endosymbiotic *Symbiodinium* cells. In the free-living cultures, *Symbiodinium* cells were in contact with the environment directly. The cells slowly proliferated, and the morphology changed due to a lower temperature or upon nitrogen deprivation (Jiang et al., 2014; Pasaribu et al., 2015). When the temperature went up above 30°C, the free-living cultures of *Symbiodinium* cells did not change in terms of the growth during the nitrogen deprivation (Fig. 2). Synergistic effects of temperature and nitrogen source on cell morphology in the *Symbiodinium* cells were examined using transmission electron microscopy (TEM) (Fig. 4). After 5 days of culture in the nitrogen-deprived medium, the cell walls of *Symbiodinium* cells were thickened, and the lipid droplets (the major organelle) occupied most of the space in the cells (Fig. 4A and B). Lipid droplet formation was found in *Symbiodinium* cells with lipid accumulation in nitrogen-deprived conditions (Jiang et al., 2014). Lipid droplet accumulation in algae typically occurs during periods of environmental stress, including growth under nutrient-deficient conditions. Nitrogen-deprivation invariably caused a steady decline in the rate of cell growth. When algal cell divisions slow down, the synthesis of new membrane components might also slow down, and the cells transform fatty acids into triacylglycerols

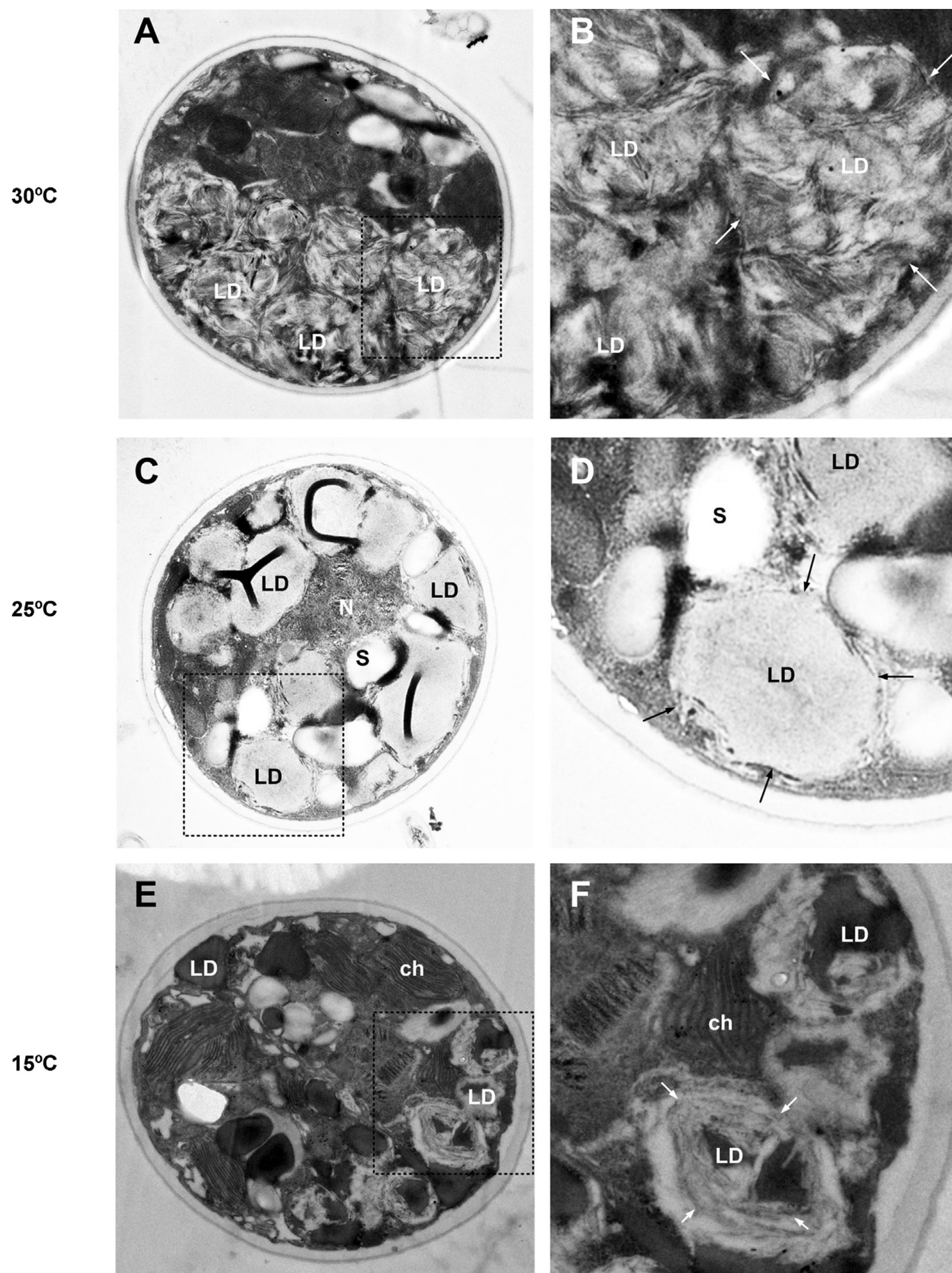

Figure 4 Morphology of *Symbiodinium* cells using transmission electron microscopy (TEM). (A and B) Ultrastructure of free-living *Symbiodinium* cells cultivated at 30°C using TEM. Free-living *Symbiodinium* cells cultivated at extreme temperatures (C and D: 25°C; E and F: 15°C). Abbreviations: LD, lipid droplet; Ch, chloroplast; N, nucleolus; S, starch granule.

(Jiang et al., 2014; Lin et al., 2012; Wang et al., 2009). Extreme temperature stress of low and high temperatures also induced lipid droplets in *Symbiodinium* cells (Fig. 4C and E). However, numerous inclusion bodies (see the arrowhead in Fig. 4D and F) appeared inside the lipid droplets on day 5 of the nitrogen-deprivation treatment, which were not found in the normal temperature treatment (Fig. 4B). The surface of lipid droplets in *Symbiodinium* cells seemed to be modified, suggesting that they might become relatively unstable. Bleaching induced similar morphological changes in *Symbiodinium* and its host (Dunn et al., 2007; Fujise et al., 2014). It was presumed that under extreme temperatures, *Symbiodinium* used lipids earlier to survive the environmental stress.

Acknowledgments

This work was supported by a grant from the Ministry of Science and Technology, Taiwan (MOST 104-2313-B-291-001 to P.-L. Jiang) and by intramural funding from NMMBA (1051016379).

References

Bajguz, A., 2009. Brassinosteroid enhanced the level of abscisic acid in *Chlorella vulgaris* subjected to short-term heat stress. J. Plant Physiol. 166 (8), 882—886.

Baker, D.M., Andras, J.P., Jordán-Garza, A.G., Fogel, M.L., 2013. Nitrate competition in a coral symbiosis varies with temperature among *Symbiodinium* clades. ISME J. 7 (6), 1248—1251.

Belda, C.A., Lucas, J.S., Yellowlees, D., 1993. Nutrient limitation in the giant clam zooxanthellae symbiosis: effect of nutrient supplement on growth of the symbiotic partners. Mar. Biol. 117 (4), 655—664.

Blank, R.J., Huss, V.A.R., 1989. DNA divergency and speciation in *Symbiodinium* (Dinophyceae). Plant Syst. Evol. 163 (3), 153—163.

Bligh, E.G., Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 37 (8), 911—917.

Converti, A.A.A., Casazza, E.Y., Ortiz, P.P., Borghi, M.D., 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chem. Eng. Process. 48 (6), 1146—1151.

Davy, K.S., Allemand, D., Weis, V.M., 2012. Cell biology of cnidarian-dinoflagellate symbiosis. Microbiol. Mol. Biol. Rev. 76 (2), 229—261.

Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: the other CO_2 problem. Annu. Rev. Mar. Sci. 1, 169—192.

Dubinsky, Z., Berman-Frank, I., 2001. Uncoupling primary production from population growth in photosynthesizing organisms in aquatic ecosystems. Aquat. Sci. 63 (1), 4—17.

Duce, R.A., LaRoche, J., Altieri, K., Arrigo, K.R., Baker, A.R., Capone, D.G., Cornell, S., Dentener, F., Galloway, J., Ganeshram, R.S., Geider, R.J., Jickells, T., Kuypers, M.M., Langlois, R., Liss, P. S., Liu, S.M., Middelburg, J.J., Moore, C.M., Nickovic, S., Oschlies, H., Pedersen, T., Prospero, J., Schlitzer, R., Seitzinger, S., Sorensen, L.L., Uematsu, M.M., Ulloa, O., Voss, M., Ward, B., Zamora, L., 2008. Impacts of atmospheric anthropogenic nitrogen on the open ocean. Science 320 (5878), 893—897.

Dunn, S.R., Schnitzler, C.E., Weis, V.M., 2007. Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: every which way you lose. Proc. R. Soc. B 274 (1629), 3079—3085.

Freudenthal, H.D., 1962. Symbiodinium gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: taxonomy, life cycle and morphology. J. Protozool. 9 (1), 45—52.

Fuchs, B., Schiller, J., Sub, R., Schurenberg, M., 2007. A direct and simple method of coupling matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) to thin layer chromatography (TLC) for the analysis of phospholipids from egg yolk. Anal. Bioanal. Chem. 389 (3), 827—834.

Fujise, L., Yamashita, H., Suzuki, G., Sasaki, K., Liao, L.M., Koike, K., 2014. Moderate thermal stress causes active and immediate expulsion of photosynthetically damaged zooxanthellae (*Symbiodinium*) from corals. PLoS One 9 (12), e114321.

Graham, L.E., Wilcox, L.W., 2000. Algae. Prentice Hall, Upper Saddle River, NJ, 640 pp.

Hastie, L.C., Waston, T.C., Isamu, T., 1992. Effect of nutrient enrichment on *Tridacna derasa* seed: dissolved in organic nitrogen increases growth rate. Aquaculture 106 (1), 41—49.

Hockin, N.L., Mock, T., Mulholland, F., Kopriva, S., Malin, G., 2012. The response of diatom central carbon metabolism to nitrogen starvation is different from that of green algae and higher plants. Plant Physiol. 158 (1), 299—312.

Hoegh-Guldberg, O., Smith, J., 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. J. Exp. Mar. Biol. Ecol. 129 (3), 279—303.

Hu, H., Gao, K., 2005. Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO_2 concentration. Biotechnol. Lett. 28 (13), 987—992.

Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., Warner, R.R., 2001. Historical overfishing and the recent collapse of coastal ecosystem. Science 293 (5530), 629—637.

Jeong, H.J., Lee, S.Y., Kang, N.S., Yoo, Y.D., Lim, A.S., Lee, M.J., Kim, H.S., Yih, W.H., LaJeunesse, T.C., 2014. Genetics and morphology characterize the dinoflagellate *Symbiodinium voratum*, n. sp. (Dinophyceae) as the sole representative of *Symbiodinium* clade E. J. Eukaryot. Microbiol. 61 (1), 75—94.

Jiang, P.L., Pasaribu, B., Chen, C.S., 2014. Nitrogen-deprivation elevates lipid levels in *Symbiodinium* spp. by lipid droplet accumulation: morphological and compositional analyses. PLoS One 9 (1), e87416.

Kinzie III, R.A., Takayama, M., Santos, S.R., Coffroth, M.A., 2001. The adaptive bleaching hypothesis: experimental tests of critical assumptions. Biol. Bull. 200 (1), 51—58.

Koop, K., Booth, D., Broadbent, A., Brodie, J., Bucher, D., Capone, D., Coll, J., Dennison, W., Erdmann, M., Harrison, P., Hoegh-Guldberg, O., Hutchings, P., Jones, G.B., Larkum, A.W.D., O'Neil, J., Steven, A., Tentori, E., Ward, S., Williamson, J., Yellowlees, D., 2001. ENCORE: the effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Mar. Pollut. Bull. 42 (2), 9—120.

Kroecker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.M., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob. Change Biol. 19 (6), 1884—1896.

Li, X., Hu, H.Y., Yang, J., 2010. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus* sp. LX1, growing in secondary effluent. New Biotechnol. 27 (1), 59—63.

Lin, I.P., Jiang, P.L., Chen, C.S., Tzen, J.T.C., 2012. A unique caleosin serving as the major integral protein in oil bodies isolated from *Chlorella* sp. cells cultured with limited nitrogen. Plant Physiol. Biochem. 61, 80—87.

Macedo, R.V.T., Alegre, R.M., 2001. Influence of nitrogen content in the cultivation of *Spirulina maxima* in two temperature levels — Part II, Produção de Lipídios. Ciênc. Tecnol. Aliment. Campinas 21 (2), 183—186.

Marubini, F., Davies, P.S., 1996. Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Mar. Biol. 127 (2), 319—328.

Mayfield, A.B., Chen, M.N., Meng, P.J., Lin, H.J., Chen, C.S., Liu, P. J., 2013. The physiological response of the reef coral *Pocillopora damicornis* to elevated temperature: results from coral reef mesocosm experiments in Southern Taiwan. Mar. Environ. Res. 86, 1—11.

Mayfield, A.B., Wang, L.H., Tang, P.C.C., Fan, T.Y., Hsiao, Y.Y., Tsai, C.L., Chen, C.S., 2011. Assessing the impacts of experimentally elevated temperature on the biological composition and molecular chaperone gene expression of a reef coral. PLoS One 6 (10), e26529.

McAuley, P.J., 1987. Nitrogen limitation and amino-acid metabolism of Chlorella symbiotic with green hydra. Planta 171 (4), 532—538.

Muscatine, L., 1980. Uptake, retention, and release of dissolved inorganic nutrients by marine alga-invertebrate associations. In: Cook, C.B., Pappas, P.W., Rudolph, E.D. (Eds.), Cellular Interactions in Symbiosis and Parasitism. Ohio Univ. Press, Columbus, 229—244.

Muscatine, L., Porter, J.W., 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27 (7), 454—460.

Nitschke, M.R., Davy, S.K., Cribb, T.H., Ward, S., 2015. The effect of elevated temperature and substrate on free-living *Symbiodinium* cultures. Coral Reefs 34 (1), 161—171.

Oku, H., Yamashiro, H., Onaga, K., 2003. Lipid biosynthesis from [14C]-glucose in the coral *Montipora digitata*. Fish. Sci. 69 (3), 625—631.

Oliveira, M.A.C.L., Monteiro, M.P.C., Robbs, P.G., Leite, S.G.F., 1999. Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. Aquacult. Int. 7 (4), 261—275.

Pasaribu, B., Lin, I.P., Chen, C.S., Lu, C.Y., Jiang, P.L., 2014. Nutrient limitation in *Auxenochlorella protothecoides* induces qualitative changes of fatty acid and expression of caleosin as a membrane protein associated with oil bodies. Biotechnol. Lett. 36 (1), 175—180.

Pasaribu, B., Weng, L.C., Lin, I.P., Camargo, E., Tzen, J.T.C., Tsai, C. H., Ho, L.S., Lin, M.R., Wang, L.H., Chen, C.S., Jiang, P.L., 2015. Morphological variability and distinct protein profiles of cultured and endosymbiotic *Symbiodinium* cells isolated from *Exaiptasia pulchella*. Sci. Rep. 5, article no. 15353.

Peng, S.E., Chen, C.S., Song, Y.F., Huang, H.T., Jiang, P.L., Chen, W. N., Fang, L.S., Lee, Y.C., 2012. Assessment of metabolic modulation in free-living versus endosymbiotic *Symbiodinium* using synchrotron radiation-based infrared microspectroscopy. Biol. Lett. 8 (3), 434—437.

Piorreck, M., Baasch, K.H., Pohl, P., 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry 23 (2), 207—216.

Poloczanska, E.S., Brown, C.J., Sydeman, W.J., Kiessling, W., Schoeman, D.S., Moore, P.J., Brander, K., Bruno, J.F., Buckley, L.B., Burrows, M.T., Duarte, C.M., Halpern, B.S., Holding, J., Kappel, C.V., O'Connor, M.I., Pandolfi, J.M., Parmesan, C., Schwing, F., Thompson, S.H., Richardson, A.J., 2013. Global imprint of climate change on marine life. Nat. Clim. Change 3, 919—925.

Powell, N.A., Shilton, A.N., Pratt, S., Chisti, Y., 2008. Factors influencing luxury uptake of phosphorus by microalgae in waste stabilization ponds. Environ. Sci. Technol. 42 (16), 5958—5962.

Rahav, O., Dubinsky, Z., Achituv, Y., Falkowski, P.G., 1989. Ammonium metabolism in the zooxanthellate coral *Stylophora pistillata*. Proc. R. Soc. Lond. B: Biol. Sci. 236 (1284), 325—337.

Rees, T.A.V., 1989. The green hydra symbiosis and ammonium II. Ammonium assimilation and release by freshly isolated symbionts and cultured algae. Proc. R. Soc. B 235 (1281), 365—382.

Rodrigues, L.J., Grottoli, A.G., 2007. Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnol. Oceanogr. 52 (5), 1874—1882.

Rowan, R., Powers, D.A., 1991. A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbiosis. Science 251 (4999), 1348—1351.

Sammarco, P.W., Strychar, K.B., 2013. Responses to high seawater temperatures in zooxanthellate octocorals. PLoS One 8 (2), e54989.

Saxby, T., Dennison, W.C., Hoegh-Guldberg, O., 2003. Photosynthetic responses of the coral *Montipora digitata* to cold temperature stress. Mar. Ecol.-Prog. Ser. 248, 85—97.

Steen, R.G., 1986. Evidence of heterotrophy by zooxanthellae in symbiosis with *Aiptasia pulchella*. Biol. Bull. 170 (2), 267—278.

Steen, R.G., Muscatine, L., 1987. Low temperature evokes rapid exocytosis symbiotic algae by a sea anemone. Biol. Bull. 172 (2), 246—263.

Stimson, J., 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J. Exp. Mar. Biol. Ecol. 153 (1), 63—74.

Strychar, K.B., Coates, M.C., Scott, P.W., Piva, T.J., Sammarco, P.W., 2005. Loss of symbiotic dinoflagellates (Symbiodinium; zooxanthellae) from bleached soft corals *Sarcophyton*, *Sinularia*, and *Xenia*. J. Exp. Mar. Biol. Ecol. 320 (2), 159—177.

Szmant, A.M., 2002. Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? Estuar. Coast. Shelf Sci. 25 (4), 743—766.

Szmant-Froelich, A., Pilson, M.E.Q., 1984. Effects of feeding frequency and symbiosis with zooxanthellae on nitrogen metabolism and respiration of the coral *Astrangia danae*. Mar. Biol. 81 (2), 153—162.

Wang, Z.T., Ullrich, N., Joo, S., Waffenschmidt, S., Goodenough, U., 2009. Algal lipid bodies: stress induction, purification, and biochemical characterization in wild-type and starchless *Chlamydomonas reinhardtii*. Eukaryot. Cell 8 (12), 1856—1868.

Weng, L.C., Pasaribu, B., Lin, I.P., Tsai, C.H., Chen, C.S., Jiang, P.L., 2014. Nitrogen limitation induces lipid droplet accumulation and alters fatty acid metabolism in symbiotic dinoflagellates isolated from *Aiptasia pulchella*. Sci. Rep. 4 article no. 5777.

Wiedenmann, J., D'Angelo, C., Smith, E.G., Hunt, A.N., Legiret, F.E., Postle, A.D., Achterberg, E.P., 2013. Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nat. Clim. Change 3, 160—164.

Wilkinson, C., 2008. Status of Coral Reefs of the World: 2008. GCMN, Reef Rainforest Res. Centre, Townsville, 290 pp.

Wooldridge, S.A., 2010. Is the coral-algae symbiosis really mutually beneficial for the partners? Bioessays 32 (7), 615—625.

Wooldridge, S.A., 2014. Assessing coral health and resilience in a warming ocean: why looks can be deceptive. Bioessays 36 (11), 1041—1049.

Yeesang, C., Cheirsilp, B., 2011. Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. Bioresour. Technol. 102 (3), 3034—3040.