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Role of organic matter in chemical symbiosis at coral reefs: release of organic nitorgen and amino acids under heat stress

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Abstract. Coral Reefs are declining worldwide due to bleaching and diseases. Biogeochemical role and mechanisms of behavior of dissolved organic matter remain unexplained on this context. Here we discuss the changes of dissolved organic nitrogen by composition of amino acids as a model study, to demonstrate linkage of coral stress to elevated temperature and behavior of chemical substances in the coral biological system. Results suggest that high concentrations of nutrients and organic matters are due to the materials release by coral organism under the elevation of water temperature. This indicates observed organic nitrogen such as amino acids, peptides, proteins released as response to coral thermal stress. C: N and N: P ratio of coral mucus of thermal stressed and initial shows that thermal stress increases production of organic nitrogen and connected to increase of total amino acid could be peptides or proteins. This is shown by total hydrolyzed amino acids released from coral under the heat stress contains mix of peptides or proteins. However, to determine and confirmed source of organic nitrogen within this context need further experiments integrating microbial sources and processes. We propose to include chemical perspective in the coral biological system by the concept of chemical symbiosis.

Key words: TOC, TON, TOP, Amino Acids, Coral Reefs, Chemical symbiosis.

Introduction

Coral reefs are declining at alarming rate worldwide, bleaching and immerging diseases are recorded for the degradation of coral reefs. Coral bleaching damages of the symbiotic zooxanthellae are presently believed to result largely from elevated temperature of the seawater and the increase of UV radiation (Hoegh-Guldberg 1999, Glynn and Crox 1990, Jokiel and Brown 2004). Mild or moderate bleaching is commonly followed by recovery, but the long-term effects of high temperature of seawater will end in an irreversible bleaching and massive death of corals. As an adverse effect of rising up seawater temperature, coral animal undergo stress, response to this stress, corals release high amounts of organic matters as mucus and ammonia (Krupp 1984). The composition of coral mucus contains variable of macromolecular compounds (Ducklow and Mitchell 1979). This suggests that organic matters in dissolved (DOM) and particulate (POM) forms might play an important role for the fate of corals and coral ecosystem (Suzuki et al. 2000 and Casareto et al. 2000) especially for the resistance of coral under the heat stress. The production of organic matters by corals can act as a mechanism for defense but also it is used in feeding for particles capture strategy. High amount of ammonia in the reefs surrounding waters can enhance the growth of pico-phytoplankton (Casareto et al. 2004, Charpy 2004), which acts as main food source for benthic community. However organic matters may enhance the growth of certain bacteria, which are known to infect corals and induce bleaching and diseases (Kline et al. 2006). Therefore release of organic matter play an important role in biogeochemical cycles of coral reef ecosystem. The knowledge from this prospective is needed further to explain the sources and utilization of organic matter cycling in coral reefs to describe coral stress and health. Dissolved organic nitrogen (DON) is that subset of the dissolved organic matter (DOM) that contains nitrogen. Large part of nitrogen compounds in coral reef waters are found as DON, while inorganic nitrogen concentration is very less in coral reef waters, because coral reef is located in the oligotrophic area. But this is “paradox” for understanding of high productivity and bio-diversity of coral reef. Most of DON is consisted of peptide, protein, amino acids, urea, etc, behavior of these compounds can influenced by coral stress due to sea temperature rise explained in global warming. DON is a heterogeneous mixture of compounds with various time scales from days to hundred years as refractory substances. A large number of compounds have been identified within the DON pool, including dissolved combined amino acids (DCAA) and dissolved free amino acids (DFAA). When stress has been given coral by increasing water temperature, light intensity and others, coral releases mucus as defense against invasion by bacteria and/or virus. It is well known that most of mucus is accepted as carbohydrate and lipid which are DOC, and less protein (Coffroth 1990). However there are very few reports on the DON and protein in mucus. Here we report the behavior of dissolved organic nitrogen and amino acids concentration; distribution and changes in its characteristics (composition of amino acids and molecular weight) elevated water temperature from field and incubation experiments with suggestions link the coral stress and chemical behavior of substances within coral biological system and reef as a whole.

Material and Methods

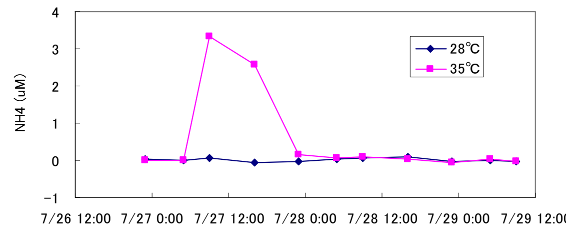
Experiments of heat stress: Experiments were done at the Sesoko Laboratory of Tropical Biosphere Research Center, Ryukyu University, Okinawa, 2006 Japan Fixed 10 branches of *Montipora digitata* specimen collected around Sesoko Island in 400 ml tank. Tanks were filled with filtration water with double filters of GFF and water was circulated with 20 ml min.$^{-1}$ from tank with 100 L to the same tank. All experimental vessels were kept under the water bath with 28^0 and 35^0, respectively. Light intensity was adjusted to 400uM with switch on and off in 12hours interval.

Measurements: Mucus samples for measurements were taken at every day during the times as 5:00, 9:00, 16:00, 23:00, for measurement for TON (total organic nitrogen) and TOP (total organic phosphorus) using an Auto-Analyzer (Braun-Lube) after digestion with alkaline persulfate, nutrients (nitrate, nitrite, ammonia, phosphate) using an Auto-Analyzer (Braun-Lube), amino acids using HPLC(Agilent) and Total Organic Carbon (TOC) by TOC 5000 (Shimadsu).

Results

Variation of inorganic nutrient concentrations recorded for temperature 28^0C and 35 ^{0}C during the experiment (Fig. 1). Also variations of Total Organic Nitrogen (TON) and Total Organic Phosphate (TOP) Concentrations are shown (Fig.2). High concentrations of ammonia, phosphate, TON and TOP were found significantly in samples of 35^0C at 9:00 a.m. of 27, July (Fig.2) Ammonia concentration increased to 3.6µM from 0.04µM with a couple of 100 times. Organic matters concentrations also increased to 29.9µM from3.3µM for TON with 10times and to 0.22µM from0.03µM for TOP.

(a)



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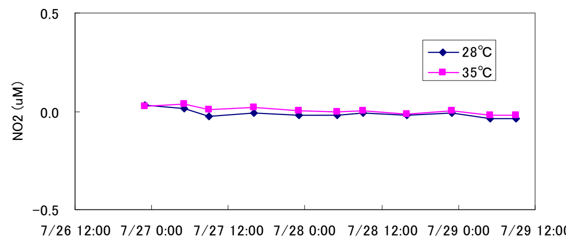

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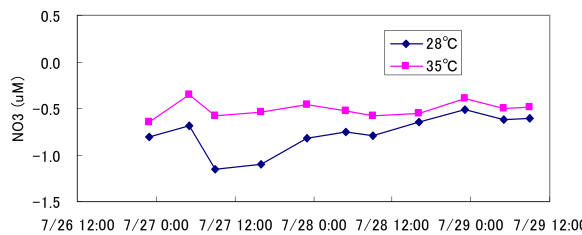

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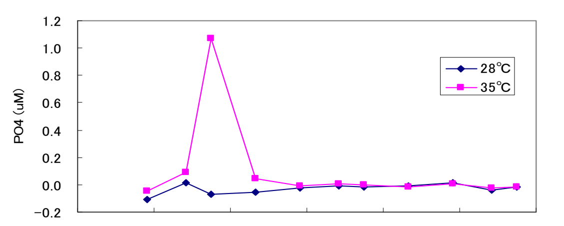
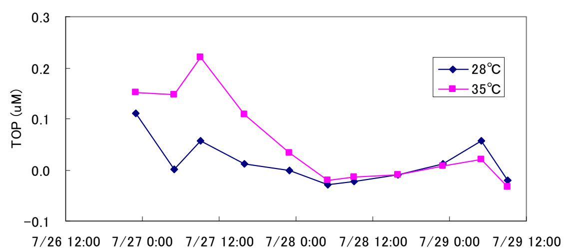


Figure 1: Variation of difference [Δ = (final) - (initial)] in Inorganic nutrient Concentrations (a)Δ Ammonium, (b) Δ Nitrite (c) Δ Nitrate and (d) Δ

Phosphate during the incubation experiments for 28^0C and 35^0C

Table 1 shows change of TOC, TON and TOP concentrations at 35^0 and 28^0C compared with initial concentrations. It was observed that increment of TON (about 10 times) is greater than that of TOC (about 3 times) at 35^0C. In Table 2 shows the Total Hydrolysis combined Amino Acids (THAA) concentrations, % of amino acids for TOC and TON. THAA concentrations increased to 414μg l^{-1}

(a)



(b)

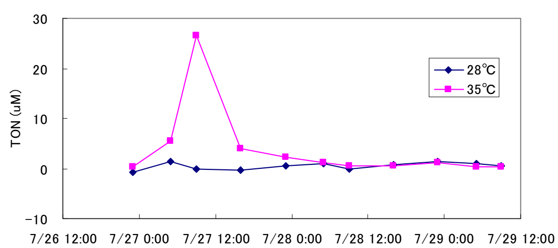


Figure 2: Variation of Concentration for Dissolved Organic (a) Nitrogen and (b) Phosphate during the incubation experiments for 28 ^{0}C and 25^0C

from12μg l^{-1} with 20 times at 35^0**,** increment rate of THAA was 7.7% for TOC and 13.6% for TON, For understanding characteristics of THAA, we divided into the high molecular weight (more than 1000 Dalton) and low molecular weight (less than 1000 Dalton) using cross flow filtration (ultra filtration techniques) for samples measured THAA. Table 3 shows organic nitrogen and THAA concentration were greater in the high molecular weight fraction for 35^0 than those for 28^0,

Table 1: (a) Mean concentration and standard deviations of Dissolved Organic Carbon and Dissolved Organic Nitrogen and (b) C/N and N/P ratio for the temperatures 28^0C and 35^0C (n=5) with one initial (In)

(a)

Date and Time		Concentration μM TOC	TON	TOP
27 Jul.	28℃	57.7 ±3.4	3.3 ±0.5	0.06 ±0.04
9:00	35℃	187.4 ±56.1	29.9 ±12.6	0.22 ±0.13
	In	60.7	3.4	nd[b]
28 Jul.	28℃	67.2 ±4.4	3.3 ±0.2	0.09 ±0.03
9:00	35℃	66.2 ±3.8	4.0 ±0.4	0.10 ±0.01
	In	59.2	3.4	0.11

(b)

Date	Exp.	Ratio C/N	N/P
27 Jul.	28℃	18 ±2.6	49 ±11
9:00	35℃	7 ±1.5	150 ±58
	In	18	nd
28 Jul.	28℃	20 ±0.7	43 ±20
9:00	35℃	17 ±2.4	41 ±6.7
	In	17	30

a: not detected

Table 2 : Mean concentration changes of Amino Acids and percentages of amino acid carbon to TOC (%AA-C) and amino acid nitrogen to TON for the temperatures 28^0C and 35^0C (n=5) with one initial (In)

Date, Time and Conditon		THAA μg l^{-1}	%AA-C	%AA-N
27 Jul.	28℃	25 ±4.7	1.5 ±0.3	7.5 ±0.7
9:00	35℃	414 ±153	7.7 ±1.6	13.6 ±1.5
	In	12	0.6	3.6
28 Jul.	28℃	54 ±12	2.8 ±0.4	16.3 ±2.7
9:00	35℃	70 ±4.2	4.3 ±1.4	18.0 ±1.3
	In	29	1.7	9.1

Further concentrations and composition of THAA during the experiments shows that Glycine and Proline represented higher concentrations. However in higher temperature increment of Proline concentration is much higher (Fig. 3).

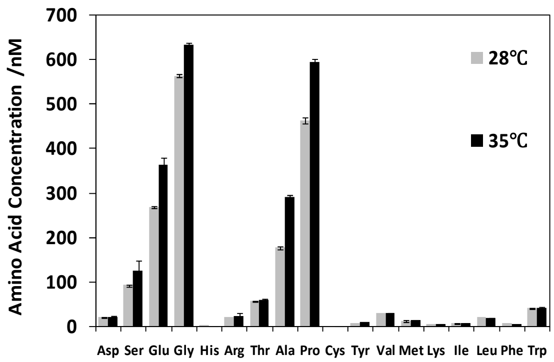


Figure 3: Variation of Concentration of the Composition of Amino Acids after two days experiments performed at 28 ^{0}C and 35^{0}C

Discussion

This experiment demonstrates new information on DON not previously reported and point's importance of linkage between coral stresses under the elevated water temperature for release of chemical substances. Results also suggest that high concentrations of nutrients and organic matters are originated by coral stress as elevation of water temperature. This indicates that organic nitrogen such as amino acids, peptides, proteins released as stress response. Considering stressed condition C: N ratio with 7 is lower and N: P ratio with 150 is higher compared with the initial samples. This observation argues that higher organic nitrogen corresponds to increase of total amino acid may be representative of peptides and proteins. Also the THAA released from coral under the heat stress supports that this possibly be peptides and or proteins. This demonstrates the considerable increment of the concentration of Proline in higher temperature could be a good representative for coral heat stress within the DON pool. However, for determination the source of organic nitrogen required further more research including microbial component. We also observed increment of bacterial abundance (Unpublished data Casareto and Suzuki), bacteria cell wall contain peptidoglycan as membrane and proteins. Future experiments will be more comprehensive after including Chemical exchange within water column, microbial component and coral biological system. We propose to include more chemical perspective in the coral biological system find the dynamics of chemical cycling by introducing the concept of chemical symbiosis within the coral animal. A simple schematic diagram in Fig. 4 explains the basic concept of chemical Symbiosis.

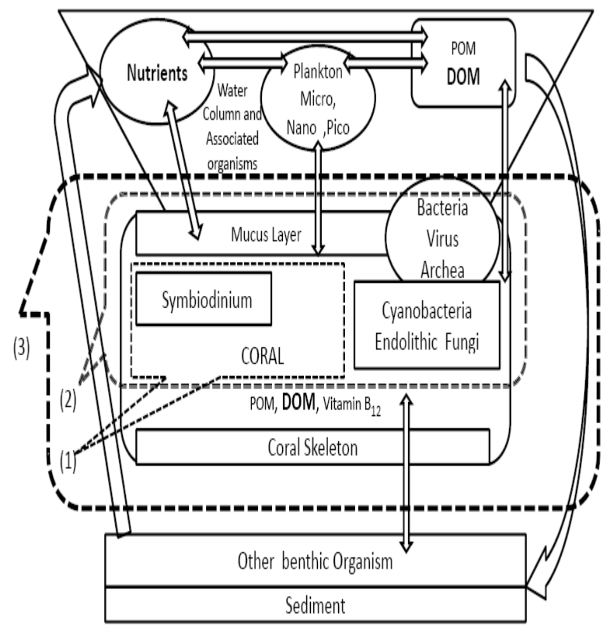


Figure 4: Proposed conceptual illustration for the chemical symbiosis with connection to detection of environment stress and physiological change in coral biological system in chemical prospective. Highlighted words reflect results to this paper. The cages represent concepts of (1) biological symbiosis, (2) holobiont and (3) chemical symbiosis.

Coral Symbiosis is well explained in coral biology and widely used in literature to show the coral animal and zoxanthellae relationship in hermatypic corals (Muscatine et al. 1984) and in microbial prospective coral system also explained as coral holobiont (Rohwer et al., 2002). However there are similarities and some differences with our proposed chemical symbiosis concept based on presentation of the idea from different perspective. Proposed concept for chemical symbiosis describes the exchange of chemical substances such as organic material, nutrients and vitamin B_{12} (Agostini et al., unpublished) essentials for the living coral biological system and production of non living organic matrix represent as coral skeleton. Also the concept of chemical symbiosis attempt to describe environmental stress and its physiological response in the view point of exchange chemicals and this chemical signals can represents the physiological indicator either in environment or in coral organism. This is needed to reveal new insight of coral reef biogeochemical cycles.

Acknowledgement

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