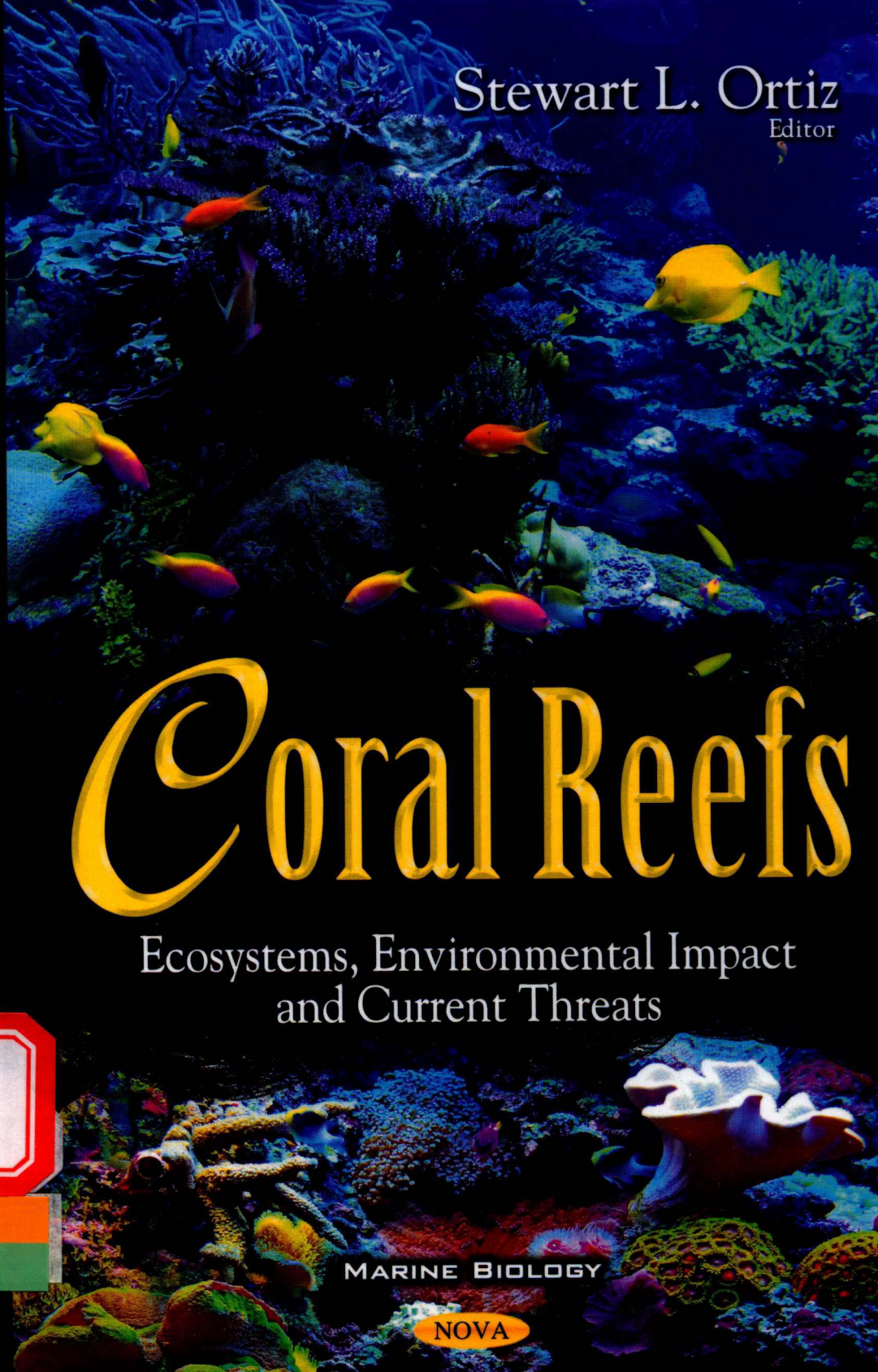
Stewart L. Ortiz
Editor
Coral Reefs
Ecosystems, Environmental Impact
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CORAL REEFS

ECOSYSTEMS, ENVIRONMENTAL IMPACT AND CURRENT THREATS

STEWART H. ORTIZ
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PREFACE

This book provides current research on the ecosystems of coral reefs, as well as the environmental impact and current threats to the coral reefs. Chapter One studies proteins responsive to variable temperature exposure in the reef-building coral *Seriatopora hystrix*. Chapters Two and Three examine the impact of arachidonic acid's (ArA) on coral reefs. The final chapter discusses environmental monitoring in the Gulf of Thailand, and the use of coral skeletons as metal pollution recorders.

Chapter 1 – Although reef-building corals engaged in mutualistic relationships with dinoflagellates of the genus *Symbiodinium* are threatened by global climate change, many anthozoan-dinoflagellate endosymbioses display a marked capacity for acclimation with respect to temperature changes. For instance, specimens of the Indo-Pacific reef coral *Seriatopora hystrix* from Southern Taiwan were found to readily acclimate to temperatures that fluctuated from 23 to 29ºC over six hours, a periodicity aimed to simulate local upwelling events that are common during boreal summer spring tides. To gain greater insight into the molecular mechanisms underlying this ability to acclimate to a variable temperature regime, proteins from corals exposed to both stable (26ºC) and variable temperatures for one week were electrophoresed across two dimensions, and differentially expressed proteins were sequenced with mass spectrometry. Seventy-five (64%) and forty-two (36%) proteins were expressed at higher levels by coral hosts and their *Symbiodinium* populations, respectively, of the stable temperature treatment. This suggests that a number of cellular pathways, including lipid body stabilization and metabolism in the *Symbiodinium* cells, are down-regulated upon exposure to variable temperature, and the potential shift in energy modulation implied by these findings may play a role in the restoration of

homeostasis necessitated by exposure to such highly variable temperature conditions.

Chapter 2 – The supply of wild fry of coral reef fishes for aquaculture has resulted in the deterioration of their natural stock status, causing public concern. Through a series of studies on the establishment of artificial-fry production technologies for coral reef fishes, the authors found that ovary, testis, eggs and fry of coral reef fishes have high or intermediate levels of arachidonic acid (ArA), which is a relatively minor component in temperate and cold-water species. In gonadal polar lipids of selected coral reef, in particular demersal fishes (19 species), ArA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) levels ranged from 6.0% to 19.4%, from 0.9% to 6.2%, and from 7.9% to 27.8%, respectively. It is notable that the major highly unsaturated fatty acids (HUFA) of polar lipids in all coral reef fish gonads are DHA and ArA (not EPA) in a ratio of about 2:1. This result allowed authors to speculate that not only DHA but also ArA may be nutritionally much important for egg development and larval growth in coral reef fishes. Thus, feeding trials were conducted to investigate the effects of dietary ArA supplementation on reproductive performance of coral reef rabbitfish (*Siganus guttatus*) broodstock. The number of spawning and the number of hatched larvae tended to be better in broodstock fed diets with ArA than in those fed a diet without ArA. Next, larval rearing tests were conducted to investigate survival and growth in rabbitfish fry fed live rotifers which had been enriched with or without ArA. Fry fed the rotifers enriched with a combination of DHA Protein Selco (Inve Aquaculture, Baasrode, Belgium) + 5% ArA (VEVODAR CRUDE ARACHIDONIC OIL, DSM Food Specialties, Delft, the Netherlands) showed significantly the best survival (44.4 ± 4.5% for Day17 fry), although growth was not different among treatments. The present study indicates that ArA is not a minor component in coral reef fishes, and that dietary ArA is very promising for the improvement of fry production technologies of the coral reef fishes.

Chapter 3 – Fatty acid composition was determined in seven species of seaweed, four species of seagrass, 17 species of invertebrates and dugong (mammal) sampled in coral reef areas in the Philippines. The data obtained indicated that Arachidonic acid (ArA) was not a minor component, and ArA distributes widely in coral reef organisms. Seagrass had high linoleic acid and linolenic acid levels with low ArA, EPA and DHA levels, while some species of seaweed had intermediate or high ArA levels (5% to 12%). In starfish, sea cucumber and some species of corals, ArA was the first major fatty acid (20% to 30%), but DHA levels were very low. Bivalves, abalone and shrimps had

intermediate ArA levels. Total lipids of abdominal muscle and liver of dugong had respectively ArA levels of 7.8% and 11.0%, which were higher than EPA levels (2.4% and 1.6%), but DHA levels (0.4% and 2.3%) were low. It is clear that ArA is a major fatty acid in coral reef animals. Thus, intermediate or high ArA levels appear to be universally found in coral reef animals. However, the origin of ArA is not still clear. Micro-organisms on the bottom or in the soil and/or macro-algae may be the sources. Although it is highly speculative, the present results suggest that the existence of an ArA-rich food chain may be widespread in coral reef areas, and that the widespread existence of ArA-rich food chain may lead to intermediate or high ArA contents in coral reef species. This speculation does not rule out the possibility that coral reef animals might have the ability to convert linoleic acid to ArA.

Chapter 4 – Coral skeletons provide useful information on aquatic environments in which corals grew, and they also offer to use as recorders of pollution history from urbanized hinterlands to coral reefs. In order to monitor pollutant discharges from urban areas to the Gulf of Thailand since the 1980s, the authors collected *Porites* corals from Khang Khao Island about 50 km southeast of Bangkok in 1985, 1998, 2001 and 2008. The coral collection periods since the 1980s coincided with a series of laws enacted by the Thai government to curb environmental pollution. To determine the skeletal growth of the samples, oxygen isotopes ($^{18}O/^{16}O$ as $\delta^{18}O$) in coral aragonite was measured by stable isotope mass spectrometry. A cyclical change in $\delta^{18}O$ is observed to record an annual change in seawater salinity, and then the coral growth rate is estimated at ~18 mm/year on average. Using the skeletal $\delta^{18}O$ method, the coral chronology was established in the Gulf of Thailand. Next the authors used a recently developed laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) method to assess the impact of metal pollution on coral skeletons taken from the Gulf of Thailand since the 1980s. The extent of anthropogenic contribution by riverine input to the gulf, including aerosol deposits, was assessed by comparing metal-calcium (Me/Ca) ratios of Khang Khao corals to those of Rukan-sho, a relatively unpolluted coral reef, Okinawa. In this comparison, high riverine inputs of Ba, V, Cd and Pb were observed from the Me/Ca values in the Thai coral samples. Since Ba concentration in seawater around Khang Khao Island largely depends on input from the rivers, especially the Chao Phraya River, the Ba/Ca ratios in Khang Khao corals reflect by the high concentrations of riverine input, showing cyclical variations like those of oxygen isotopes in the coral samples. The V/Ca ratios of Khang Khao corals showed a higher average value than that of the Rukan-sho coral, suggesting anthropogenic vanadium inputs due to fuel oil

pollution in the Gulf since the late 1990s. Higher Cd/Ca ratios were observed in Khang Khao corals compared to that of Rukan-sho coral, indicating that the Cd concentration in the Gulf continuously suffered from anthropogenic input since 1983. The levels of Cd in the coral indicate a gradual decrease in the Gulf in the late 1990s, with a drastic drop in concentration from the 1980s. The historical variation in Pb/Ca ratios recorded in the coral skeletons suggests the Gulf of Thailand suffered from anthropogenic lead from 1985 to 2001. The Pb/Ca values recorded in Khang Khao Island corals suggest that the Gulf has been polluted by anthropogenic Pb from the early 1990s. After the use of leaded gasoline was banned in Thailand since 1995, the Pb/Ca in the Khang Khao Island corals showed a remarkable decrease, indicating that regulatory control has limited anthropogenic Pb inputs to the Gulf of Thailand. In conclusion, the coral archival record of the metals (V, Cd, Hg and Pb) strongly suggests the success of the environmental laws and regulations by the Thai Government introduced since the middle 1990s.

CONTENTS

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Chapter 1

PROTEINS RESPONSIVE TO VARIABLE TEMPERATURE EXPOSURE IN THE REEF-BUILDING CORAL *SERIATOPORA HYSTRIX*

Anderson B. Mayfield[1,2,3,*], Yi-Jyun Chen[2,3], Chi-Yu Lu[4,5] and Chii-Shiarng Chen[1,2,6,7]

[1]Living Oceans Foundation, Annapolis, MD, US
[2]National Museum of Marine Biology and Aquarium, Checheng, Pingtung, Taiwan, R.O.C.
[3]Taiwan Coral Research Center, Checheng, Pingtung, Taiwan, R.O.C.
[4]Department of Biochemistry, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.
[5]Center for Research Resources and Development, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.
[6]Graduate Institute of Marine Biotechnology, National Dong-Hwa University, Checheng, Pingtung, Taiwan, R.O.C.
[7]Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung, Taiwan, R.O.C.

* Author to whom correspondence should be addressed email: andersonblairmayfield@gmail.com; Tel.: + 1-337-501-1976; Fax: +886-2-265-18660.

ABSTRACT

Although reef-building corals engaged in mutualistic relationships with dinoflagellates of the genus *Symbiodinium* are threatened by global climate change, many anthozoan-dinoflagellate endosymbioses display a marked capacity for acclimation with respect to temperature changes. For instance, specimens of the Indo-Pacific reef coral *Seriatopora hystrix* from Southern Taiwan were found to readily acclimate to temperatures that fluctuated from 23 to 29ºC over six hours, a periodicity aimed to simulate local upwelling events that are common during boreal summer spring tides. To gain greater insight into the molecular mechanisms underlying this ability to acclimate to a variable temperature regime, proteins from corals exposed to both stable (26ºC) and variable temperatures for one week were electrophoresed across two dimensions, and differentially expressed proteins were sequenced with mass spectrometry. Seventy-five (64%) and forty-two (36%) proteins were expressed at higher levels by coral hosts and their *Symbiodinium* populations, respectively, of the stable temperature treatment. This suggests that a number of cellular pathways, including lipid body stabilization and metabolism in the *Symbiodinium* cells, are down-regulated upon exposure to variable temperature, and the potential shift in energy modulation implied by these findings may play a role in the restoration of homeostasis necessitated by exposure to such highly variable temperature conditions.

INTRODUCTION

Most current global climate change (GCC) models assume that reef-building corals are unable to acclimate to changes in their abiotic environment [1]. Although it is true that many corals are known to live near the upper threshold of their thermotolerance and readily bleach in response to sustained temperature increases [2-3], recent studies have revealed that not only can corals readily acclimate to elevated temperature, salinity, and pCO_2 [4-7], but they can thrive under such conditions [8-10]. For instance, corals from Houbihu, Taiwan (Figure 1A) are readily exposed to episodic, spring tide upwelling during the boreal summer, periods during which temperatures may change up to 9-10ºC within several hours [11]. Corals from these upwelling habitats have proven to be markedly resilient to both short- [12] and long-term [13] increases in temperature, as has been predicted to occur based on studies of intertidal organisms [14].

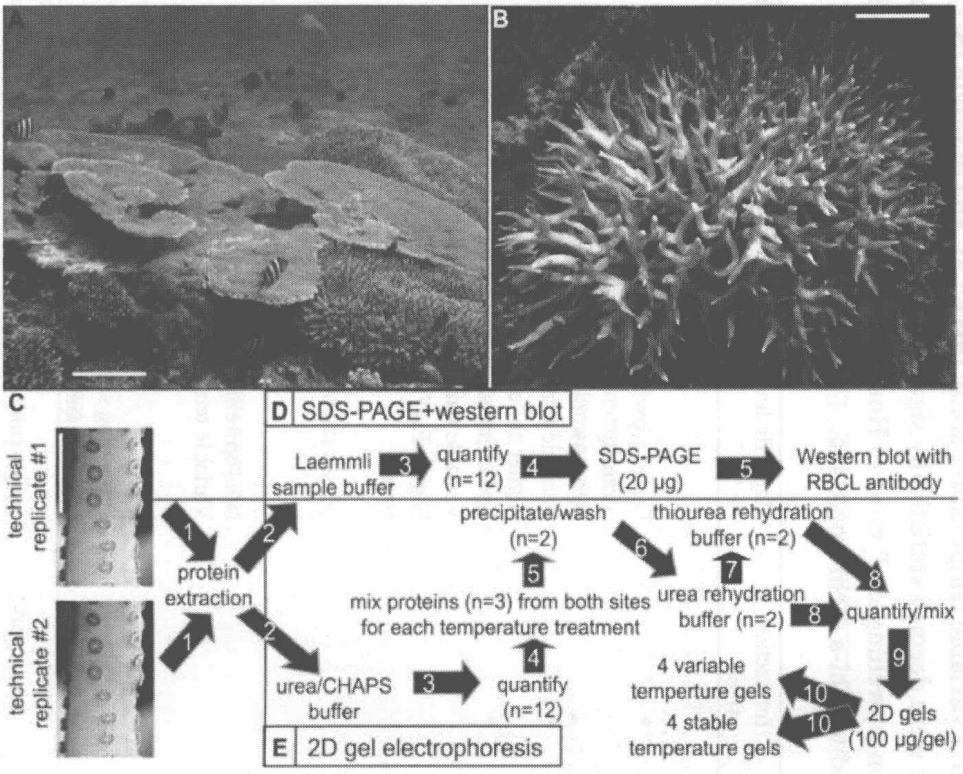

Figure 1. The upwelling field site Houbihu, the model coral *Seriatopora hystrix*, and an analytical flow-chart of the proteomic analyses. (A) Houbihu, the upwelling site from which half of the *Seriatopora hystrix* specimens used in the variable temperature study were sampled (photograph taken by Dr. Pi-Jen Liu, National Museum of Marine Biology and Aquarium, Taiwan). (B) An adult *S. hystrix* colony. (C) Proteins were extracted from each of two technical replicates (i.e., nubbins) from each of the 12 experimental aquaria after a 7-d exposure to either variable (23-29°C over a 6-hr period; n = 6 aquaria) or stable temperature (26°C; n = 6 aquaria). (D) The expression of RBCL was quantified in the 12 samples dissolved in SDS-PAGE sample buffer. (E) For the second technical replicate from each aquarium, proteins were prepared for 2-dimensional gel electrophoresis as described in the text. Proteins were pooled across sites of origin (SO; i.e., proteins from corals from Houbihu were mixed with those of corals from Houwan) for each of the two temperature treatments (TT) given the fact that *only* a TT effect on protein expression was of interest herein. The numbers on the arrows represent the respective experimental steps in E, and the scale bars in panels A, B, and C represent 500, 50, and 5 mm, respectively.

Table 1. Summary of results of the *Seriatopora hystrix* variable temperature study (SHVTS). Site of origin (SO), temperature treatment (TT), and interaction effects were deemed statistically significant at $\alpha < 0.05$ (denoted by "*" in the respective cells). "Upwelling site" and "non-upwelling site" refer to Houbihu and Houwan, respectively. NA = not applicable. Chl-a = chlorophyll a

Response variable	SO effect	TT effect	Interaction effect	Major finding(s)	Reference
Host coral genotype		NA	NA		[17]
Symbiodinium genotype					[17]
Growth			*	Faster growth in non-"transplanted" corals	[15]
Symbiodinium density	*			Non-upwelling site > upwelling site	[15]
Chl-a concentration		*	*	Variable temperature > stable temperature Higher chl-a in non-"transplanted" corals	[15]
Maximum dark-adapted quantum yield of photosystem II (F_v/F_m)	*	*	*	Upwelling site > non-upwelling site Variable temperature > stable temperature Higher F_V/F_M in non-"transplanted" corals	[15]
Symbiodinium heat shock protein 70 (*hsp70*) genome copy proportion (DNA content)					[15]
RNA/DNA ratio					[15]
Protein/DNA ratio	*			Non-upwelling site > upwelling site	[15]
Symbiodinium ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) mRNAxpression		*		Variable temperature > stable temperature	[15]
Symbiodinium photosystem I (subunit III; *psI*) mRNA expression	*	*		Upwelling site > non-upwelling site Variable temperature > stable temperature	[15]

Response variable	SO effect	TT effect	Interaction effect	Major finding(s)	Reference
Symbiodinium phosphoglycolate phosphatase (*pgpase*) mRNA expression		*		Variable temperature > stable temperature	[15]
Symbiodinium ascorbate peroxidase (*apx1*) mRNA expression					[15]
Symbiodinium hsp70 mRNA expression		*		Variable temperature > stable temperature	[17]
Symbiodinium nitrate transporter 2 (*nrt2*) mRNA expression					[17]
S. hystrix hsp70 mRNA expression		*		Stable temperature > variable temperature	[17]
S. hystrix α-tubulin (*tuba*) mRNA expression					[17]
S. hystrix tropomyosin (*trp1*) mRNA expression					[17]
S. hystrix β-actin (*actb*) mRNA expression		*		Variable temperature > stable temperature	[17]
S. hystrix ezrin mRNA expression					[17]
S. hystrix phospholipase α-2 (*cplap2*) mRNA expression			*	Higher mRNA expression in "transplanted" corals	[17]
S. hystrix transient receptor cation channel (*trcc*) mRNA expression					[17]
S. hystrix organic anion transporter (*oatp*) mRNA expression					[17]
Symbiodinium RBCL protein expression					herein
Protein expression (2D gel)		*		Stable temperature > variable temperature	herein

Table 2. A breakdown of the 10 sequenced protein spots by compartment of origin: coral host or *Symbiodinium*. Of the 117 unique peptides that were sequenced and met the minimal inclusion threshold criteria (described in the main text), 75 (64%) were from the coral host, *Seriatopora hystrix*, and 42 (36%) were from the dinoflagellate endosymbionts (genus *Symbiodinium*) living within the hosts' gastrodermal cells. Two 2-sample proportion tests were conducted to determine if one compartment (host coral or *Symbiodinium*) was over-represented in the partially sequenced proteome within each spot; for the first test, the raw proportions were compared (non-adjusted). For the second, the total number of *Symbiodinium* proteins was multiplied by 1.8 to adjust for the fact that the host contributed 75 of the 117 unique proteins (i.e., 64% host/36% *Symbiodinium* = 1.8) across all 10 spots. For the "Total/Average" row, the total number of proteins is given for the 3rd-5th columns while the average percentages are given for the "% host" and "% *Symbiodinium*" columns; error terms represent standard deviation for the latter. NS = not significant (2-sample proportion test, $p > 0.05$). NA = not applicable. kDa = kilodalton. pI = isoelectric point

Spot	Molecular weight (kDa)	pI	# host proteins	# *Symbio-dinium* proteins	# total proteins	% host	% *Symbiodinium*	2-sample proportion test *p* (non-adjusted)	2-sample proportion test *p* (adjusted)	Conclusion
1	27.4	4.9	6	7	13	46	54	NS	NS	
2	27.3	5.0	12	7	19	63	37	NS	NS	
3	27.4	5.2	14	4	18	78	22	<0.001	<0.05	Host > *Symbiodinium*
4	27.3	5.5	13	4	17	77	23	<0.01	NS	
5	20.8	4.8	16	11	27	59	41	NS	NS	
6	21.1	5.1	6	8	14	43	57	NS	<0.05	*Symbiodinium* > host
7	20.9	5.3	8	6	14	57	43	NS	NS	
8	20.7	5.3	10	8	18	56	44	NS	NS	
9	20.4	5.5	9	5	14	64	36	NS	NS	
10	20.0	5.9	6	8	14	47	53	NS	<0.05	*Symbiodinium* > host
Total/Average			100	68	168	59 ± 12	41 ± 12	NA	<0.0001	Host > *Symbiodinium*
Unique			**75**	**42**	**117**	**64**	**36**	NA	<0.0001	Host > *Symbiodinium*

In order to gain insight into how corals from these upwelling sites acclimate to such dramatic temperature changes, an experiment was conducted in which corals from not only Houbihu, but also a nearby, non-upwelling site, Houwan, were exposed to either a variable (23-29ºC over a 6-hr period) or stable (26ºC) temperature profile for seven days [15-17]. *Seriatopora hystrix* (Figure 1B-C) was chosen as the model coral for such laboratory-based studies, given its 1) widespread distribution across the Indo-Pacific [18-19], 2) propensity for bleaching under periods of elevated temperatures [20], and 3) modest existing understanding of its molecular eco-physiology [21-22]. In general, even *S. hystrix* specimens that were never exposed to upwelling *in situ* readily acclimated to variable temperature conditions (Table 1), and an effort was made to develop both a physiological and a sub-cellular understanding of how such acclimation occurred in the samples from this "*Seriatopora hystrix* variable temperature study" (SHVTS; [15-17]).

Given recent success in employing molecular biology-driven approaches to answering an array of both fundamental [23-27] and stress/environmental biology [28] questions in the field of anthozoan-dinoflagellate endosymbiosis, the expression of a series of gene mRNAs was measured in samples of the SHVTS [15, 17]. Although several genes encoding proteins involved in photosynthesis were differentially expressed between the stable and variable temperature treatments (TT; [15, 17] and Table 1), the variation was generally modest, and it was, furthermore, unclear whether such changes in mRNA expression would actually lead to altered levels of translation of the respective proteins; indeed, in the few studies that have looked at both gene and protein expression in the same anthozoan-dinoflagellate sample [7, 26], there was not always a significant, positive correlation between gene and protein expression [7]. Therefore, a whole-proteome-based approach employing two-dimensional (2D) electrophoresis followed by protein sequencing via mass spectrometry (MS) was taken herein in order to better unravel the molecular means by which *S. hystrix* and its endosymbiotic *Symbiodinium* populations acclimate to a variable temperature regime.

MATERIALS AND METHODS

SHVTS

The SHVTS was discussed in previous works [15-17]. Briefly, six *S. hystrix* colonies from both the upwelling (Houbihu; Figure 1A) and non-upwelling (control) sites (Houwan) were collected, acclimated in indoor