

OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY
GRADUATE UNIVERSITY

Thesis submitted for the degree

Doctor of Philosophy

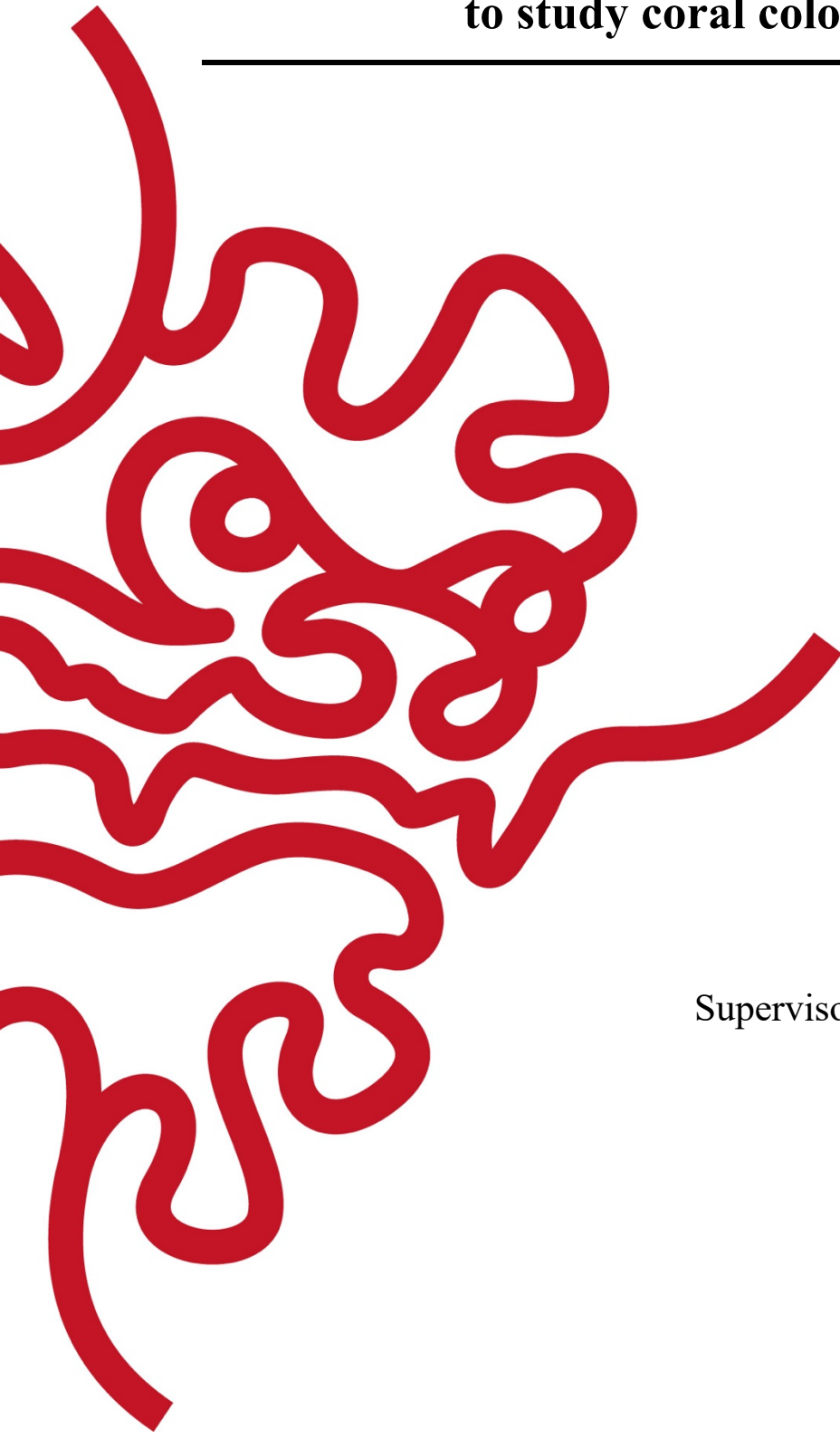
**From Polyps to Colonies: Applying polyp bail-out
to study coral coloniality**

by

Po-Shun Chuang

Supervisor: **Prof. Satoshi Mitarai**

December 1st, 2021



Declaration of Original and Sole Authorship

I, Po-Shun Chuang, declare that this thesis, entitled, *From Polyps to Colonies: Applying polyp bail-out to study coral coloniality*, and the data presented herein are original and my own work.

I confirm that:

- No part of this work has previously been submitted for a degree at this or any other university.
- The work of others has been clearly acknowledged. Quotations from the work of others are clearly indicated, and attributed to them.
- In cases where others have contributed to part of this work, such contributions have been clearly acknowledged and distinguished from my own work.
- None of this work has been previously published elsewhere, with the exception of the following:

1. **Chuang, Po-Shun, and Satoshi Mitarai.** "Signaling pathways in the coral polyp bail-out response." *Coral Reefs* 39.6 (2020): 1535-1548.

2. **Chuang, Po-Shun, Kota Ishikawa, and Satoshi Mitarai.** "Morphological and Genetic Recovery of Coral Polyps After Bail-Out." *Frontiers in Marine Science* 8 (2021): 280.

3. **Chuang, Po-Shun, and Satoshi Mitarai.** "Genetic changes involving the coral gastrovascular system support the transition between colonies and bailed-out polyps: evidence from a *Pocillopora acuta* transcriptome." *BMC genomics* 22.1 (2021): 1-12.

Date: December 1st, 2021

Signature:



Abstract

From Polyps to Colonies: Applying polyp bail-out to study coral coloniality

Colonial lifestyles have been adopted by the majority of shallow-water stony corals (Cnidaria: Scleractinia), as they facilitate coral responses to environmental changes. Polyp bail-out, a coral stress response featuring colony dissociation and polyp detachment, offers a platform to study coloniality in stony corals. However, employing bailed-out polyps in coral research requires greater understanding of the biology of this stress response. This thesis investigates the molecular basis of polyp bail-out in *Pocillopora acuta*, a branching coral that is common in the tropical waters of the Indo-Pacific Ocean, and examines bailed-out polyps to study the biological foundation of coral coloniality. First, I probe molecular mechanisms involved in hyperosmosis-induced polyp bail-out, based upon a *P. acuta* transcriptome assembly. Then, I monitor morphological and genetic changes of bailed-out *P. acuta* polyps after the induced bail-out response. Finally, I explore transcriptional profiles of bailed-out polyps and those of polyps in normal colonies in order to identify differences between corals at different levels of structural and social complexity. Based on transcriptomic data, activation of tumor necrosis factor and fibroblast growth factor signaling pathways was revealed during initiation of polyp bail-out, possibly linking these pathways to colony dissociation and polyp detachment, respectively. Under ambient conditions, about half of bailed-out polyps displayed morphological recovery and genetic resumption of fundamental cellular processes within five days. Compared with recovered bailed-out polyps, normal colonies showed activation of genes for neurological and circulatory system development. Furthermore, in response to environmental stresses, few genetic changes were shared by bailed-out polyps and colonies, suggesting that coloniality promotes distinctively different stress responses, probably enhancing fitness in stony corals. Interestingly, transcriptomic data also revealed differential expression of angiotensin-converting and endothelin-converting enzymes in the transition between normal colonies and bailed-out polyps. This thesis presents a robust polyp bail-out induction protocol and develops a foundation for its application to coral research. Using this new research model, this thesis presents the first molecular-level study of coral coloniality and identifies genes potentially participating in development of coral colonies, which are expected to be fruitful topics for future studies.

Acknowledgment

I thank Satoshi Mitarai for his invaluable support of my Ph.D. program, as both a highly reliable supervisor and a great friend. I am grateful for his consistent encouragement throughout my Ph.D. research in regard to both the execution of experiments and manuscript publication. I also appreciate his guidance in regard to career development.

I thank the Japan Society for the Promotion of Science (JSPS) for a JSPS DC1 fellowship [2018–2021, Kakenhi grant number JP18J20226], which provided support for this Ph.D. research. I also thank Okinawa Institute of Science and Technology Graduate University (OIST) for its financial support, with subsidy funding from the Cabinet Office, Government of Japan.

I greatly appreciate members of the Marine Biophysics Unit at OIST, past and present, for their valuable input and feedback on this project. Dr. Yuichi Nakajima (Nagahama Institute of Bio-Science and Technology) and Dr. Atsushi Fujimura (University of Guam) were especially generous in sharing their knowledge of corals, which greatly facilitated development of my research. I thank Mr. Kota Ishikawa for contributing to some of the experiments and to publication of Chapter 2 of this thesis. I thank Dr. Wu Heng for constructive scientific discussions. I also appreciate Ms. Kazumi Inoha and Ms. Tomoko Yoshino for their constant administrative help.

I am indebted to Mr. Toda Koichi, Dr. Nobuo Ueda, Dr. Kosuke Mori, and Dr. Yoshikatsu Nakano for helping me to build an experimental setup and for their generous support at the OIST Marine Science Station (OMSS). I thank the Sequencing Section (SQC) at OIST for support in constructing and sequencing the RNA-seq libraries reported in this thesis. I am also indebted to the Scientific Computing & Data Analysis Section (SCDA) at OIST for its generosity in providing the High-Performance Computing (HPC) service that was used to construct all the transcriptome assemblies reported in this thesis.

I thank my thesis committee members, Professor Amy Shen and Professor Ichiro Masai for their insightful comments throughout my Ph.D. career. I also thank my thesis proposal examiners, Professor Dan Tchernov and Professor Tadashi Yamamoto, for valuable suggestions about this Ph.D. project. I express my sincere appreciation to Dr. Joseph Loya and Dr. Ann Tarrant for serving as thesis examiners and for their valuable comments on it. I thank Dr. Tsung-Han Tsieh and Mr. Hung-Ju Chiang for constructive scientific discussions. I especially appreciate Dr. Steven D. Aird for editing this thesis and all published articles resulting from it. I have learned much about scientific writing from him. Lastly, I express my gratitude to my wife, Kato Mizuki, my friends, and my family for their constant encouragement throughout my time as a Ph.D. student.

Abbreviations

ACE angiotensin-converting enzyme
ASW artificial seawater
CT cycle threshold
CPM counts per million
DE differential gene expression
DEG differentially expressed gene
DIC dissolved inorganic carbon
DO dissolved oxygen
ECE endothelin-converting enzyme
ECM extracellular matrix
FDR false discovery rate
FE fold enrichment
FGF fibroblast growth factor
FSW sand-filtered seawater
GO gene ontology
MMP matrix metalloproteinase
OIST Okinawa Institute of Science and Technology
ORF Open reading frame
PC principle component
PCA principle component analysis
qPCR quantitative polymerase chain reaction
RIN RNA integrity number
SD standard deviation
TNF tumor necrosis factor
TPM transcripts per million

List of Publications

Publications and author contributions are listed in the order of appearance in the body of this thesis.

1. Chuang, Po-Shun, and Satoshi Mitarai. "Signaling pathways in the coral polyp bail-out response." *Coral Reefs* 39.6 (2020): 1535-1548.

Contributions

PSC conceptualized the research, conducted all experiments, and wrote the manuscript. SM assisted in editing the manuscript.

2. Chuang, Po-Shun, Kota Ishikawa, and Satoshi Mitarai. "Morphological and Genetic Recovery of Coral Polyps After Bail-Out." *Frontiers in Marine Science* 8 (2021): 280.

Contributions

PSC conceptualized the research idea, conducted all experiments (except for the part conducted by KI), and wrote the manuscript. KI conducted some of the qPCR experiments and helped edit the manuscript. SM assisted in editing the manuscript.

3. Chuang, Po-Shun, and Satoshi Mitarai. "Genetic changes involving the coral gastrovascular system support the transition between colonies and bailed-out polyps: evidence from a *Pocillopora acuta* transcriptome." *BMC genomics* 22.1 (2021): 1-12.

Contributions

PSC conceptualized the research, conducted all experiments, and wrote the manuscript. SM assisted in editing the manuscript.

Contents

DECLARATION OF ORIGINAL AND SOLE AUTHORSHIP.....	III
ABSTRACT	V
ACKNOWLEDGMENT	VII
ABBREVIATIONS.....	IX
LIST OF PUBLICATIONS	XI
CONTENTS	XIII
LIST OF FIGURES.....	XV
INTRODUCTION	1
IMPORTANCE OF CORAL REEFS	1
COLONIALITY IN STONY CORALS.....	2
POLYP BAIL-OUT: DESCRIPTION AND APPLICATION	3
RESEARCH AIMS	5
1. CHAPTER 1 MOLECULAR MECHANISM OF POLYP BAIL-OUT.....	7
1.1 INTRODUCTION.....	7
1.2 PUBLISHED ARTICLE.....	7
1.3 CONCLUSIONS.....	7
2. CHAPTER 2 RECOVERY OF CORAL POLYPS AFTER BAIL-OUT.....	9
2.1 INTRODUCTION.....	9
2.2 PUBLISHED ARTICLE.....	9
2.3 CONCLUSIONS.....	9
3. CHAPTER 3 GENETICS IN CORAL COLONIALITY	11
3.1 INTRODUCTION.....	11
3.2 PUBLISHED ARTICLE	11
3.3 CONCLUSIONS.....	11
CONCLUSIONS.....	13
BIBLIOGRAPHY.....	15

List of Figures

<i>Figure 1. Experimental design and objectives of this thesis. Results of each chapter are elaborated separately.</i>	<i>5</i>
---	----------

Introduction

Importance of coral reefs

Hotspots of biodiversity

Coral reefs, the “rainforests of the sea”, support the highest biodiversity among marine ecosystems (Hoegh-Guldberg 1999; Moberg and Folke 1999; Roberts et al. 2002; Carpenter et al. 2008). Comprising about 255,000 km², coral reefs cover less than 0.2% of the global ocean floor (Spalding and Grenfell 1997; Knowlton et al. 2010). However, these ecosystems show primary productivity rivaling the most productive terrestrial plant communities in the world (Sargent and Austin 1949; Hatcher 1988) and host about a quarter of known marine species (Spalding et al. 2001). Assuming that coral reefs present the same area-specific species richness as rainforests and that rainforests contain about two million species, it has been proposed that global coral reefs host a potential 950,000 species, which corresponds to about half of described global biodiversity (Reaka-Kudla 1997). However, in contrast to tropical rainforests, with their immense net primary productivity, the net output from coral reefs is surprisingly low (Crossland et al. 1991; Geider et al. 2001). This suggests a delicate balance of food webs in these ecosystems, such that slight changes in populations of primary producers may result in significant perturbations of food chains.

Economic significance to human society

Coral reefs contribute great economic value to human society in multiple regards. Abundant fish and invertebrate taxa in these ecosystems make them important fisheries for coastal tropical countries (Reaka-Kudla 1997). Reports from the late 20th and early 21st centuries estimate that over 6 million tons of fish were caught from coral reefs, corresponding to about 9% of annual global fish consumption and an estimated \$5.7 billion in economic benefit from coral reefs globally (Pac 1978; Munro 1996; Cesar et al. 2003). In addition, the economic significance of coral reefs is reflected in tourism and other socio-economic services, such as coastal protection and research value (Spurgeon 1992; Cesar 2000). Together with the fishery yield, coral reefs offer an estimated annual net benefit of about \$30 billion to human society (Cesar et al. 2003). Unquestionably, this value is underestimated since most ecological goods and services were not included in early estimates (Moberg and Folke 1999). Given the growth of the global fishery industry and advances in human technology in recent decades, the economic significance of coral reefs to human society is undoubtedly even higher.

Role in global carbon cycle

In addition to their importance to marine ecology and human economics, coral reefs participate in the global carbon cycle as long-term carbon reservoirs (Kinsey and Hopley 1991; Rees et al. 2005). Based on calcification rates of different reef types and their

distributions in the Great Barrier Reef, the average calcium carbonate precipitation rate of the Great Barrier Reef was estimated $\sim 2.4 \text{ kg/m}^2/\text{y}$ (Kinsey and Hopley 1991). With global coral reef coverage estimates ranging from 200,000 to 600,000 km^2 (Pac 1978; Spalding and Grenfell 1997; Knowlton et al. 2010), this production rate implies fixation of 0.5 – 1.4 billion tons of CaCO_3 per year by global coral reefs, corresponding to 1 – 3% of anthropogenic CO_2 (Kinsey and Hopley 1991). However, as formation of CaCO_3 from calcium and bicarbonate ions is accompanied by production of an equal amount of CO_2 , coral reefs are also sources of atmospheric CO_2 , albeit an insignificant contribution compared to anthropogenic emissions (Ware et al. 1992; Kawahata et al. 1997; Gattuso et al. 1999). The status of coral reefs as sinks or sources of atmospheric CO_2 is therefore still under debate, and may require understanding of more complex biotic and abiotic factors (Chisholm and Barnes 1998; Bates 2002).

Coloniality in stony corals

Evolution of coloniality in the Scleractinia

Owing to their susceptibility to environmental stresses, stony corals (Cnidaria: Anthozoa: Scleractinia), the major structural component of coral reefs, have received great attention from researchers in multiple disciplines. The Scleractinia includes roughly equal numbers of taxa with solitary and colonial lifestyles (Simpson 2013; Campoy et al. 2020). Based on molecular data from both mitochondrial and nuclear genes, Barbeitos et al. (2010) proposed zooxanthellate and colonial lifestyles for the ancestors of modern scleractinian corals. This accords with paleontological and isotopic evidence, which show highly developed colony integration and photosymbiosis at the first appearance of scleractinian corals in the fossil record in the middle Triassic (Stanley 1988; Stanley and Fautin 2001; Muscatine et al. 2005; Stanley Jr and Helmle 2010). However, given the immense taxonomic diversity and high level of colony integration, it is also strongly argued that the Scleractinia has an even longer evolutionary history (Zhanqiu and Lei 1984; Stanley and Fautin 2001; Stanley Jr 2003; Stolarski et al. 2011; Bo et al. 2017). Supporting this alternative hypothesis, several molecular phylogenetic studies proposed that the common ancestor of extant scleractinian corals can probably be traced back to the Paleozoic (Romano and Palumbi 1996; Romano and Cairns 2000; Stolarski et al. 2011), which was likely azooxanthellate and solitary (Campoy et al. 2020). Disregarding the debate about the ancestral lifestyle of scleractinian corals, coevolution of coloniality and photosymbiosis in the Scleractinia is hypothesized by all studies (Barbeitos et al. 2010; Campoy et al. 2020). This concurrence results in distinctive distribution patterns of both lifestyles in scleractinian corals, with colonial corals dominating shallow water reefs, and solitary species more widely distributed geographically and more abundant in deep water.

Functional advantages of coloniality in stony corals

Coloniality is considered an adaptive trait in the competition for space (Barbeitos et al. 2010; Campoy et al. 2020; Hiebert et al. 2020). Employing asexual reproduction for colonial growth enables colonial corals to colonize potential habitats in an efficient

manner in shallow water, where hard substrates for attachment are limited. The extra energy required for rapid colonial growth is likely supplied by photosynthesis of symbiotic zooxanthellae, explaining the coevolution of photosymbiosis and coloniality in stony corals (Simpson 2013). Furthermore, it has also been hypothesized that photosynthesis promotes coral calcification (Moya et al. 2006; Cohen et al. 2016), creating more habitat for growth of colonial corals; thus bundling coloniality, photosymbiosis, and calcification in stony corals. Interestingly, a recent study found that colony integration is correlated with bleaching resistance in the Scleractinia (Swain et al. 2018), illuminating discussions about other ecological functions of coloniality in stony corals.

During formation of a coral colony, an important morphological change is the development of coenosarc tissue that connects neighboring polyps. In an early study on the brain coral, *Meandrina meandrites*, coenosarc tissue was shown to constitute a neural network for inter-polyp communication (McFarlane and Callan 1978). In addition to neurological signaling, experiments using isotope labeling and fluorescent dyes also showed that molecules and cellular components can travel through coenosarc tissue within a coral colony (Gladfelter 1983; Gateno et al. 1998; Fine et al. 2002). These physiological features are thought to enable corals to respond to endogenous and external stimuli with either colony-wide or spatial-specific actions (McFarlane and Callan 1978; Roff et al. 2006). However, the biology of coral coloniality, especially from a molecular perspective, is still poorly understood.

Polyp bail-out: description and application

A novel stress response in stony corals

In recent decades, polyp bail-out has become recognized in coral research as a coral stress response. The first documentation of polyp bail-out may be that of Goreau and Goreau (1959), who stated that, “*polyps of starving corals are able to detach themselves completely from the corallum.*” However, more detailed description about this phenomenon was not offered until the early 1980s, when Sammarco (1982) reported “*an escaping response to environmental stress*” in *Seriatopora hystrix* and coined the term, “polyp-bail-out.” Based on laboratory observations, Sammarco (1982) described polyp bail-out as commencing with polyp-ward retraction of coenosarc tissue, which dissociates coral colonies into isolated polyps, followed by detachment of individual polyps from their calcareous skeletons. In contrast to coral bleaching, which disrupts the endosymbiotic partnership between corals and photosynthetic zooxanthellae to prevent accumulation of harmful molecules in coral tissues (Brown 1997; Douglas 2003), polyp bail-out is characterized by release of free coral polyps, together with their symbiotic zooxanthellae. Using carefully designed experiments and advanced techniques, more recent studies have identified coenosarc-specific programmed cell death (apoptosis) and extracellular matrix (ECM) degradation in polyp bail-out, which likely contribute to colony dissociation and polyp detachment, respectively (Kvitt et al. 2015; Wecker et al. 2018). These reports created the foundation for subsequent studies on this intriguing coral

response.

Ecological roles of polyp bail-out

In Sammarco (1982), polyp bail-out was described as finishing with re-attachment of polyps to substrates and re-initiation of calcification, leading to a hypothetical role of this stress response as a form of asexual reproduction in stony corals (Sammarco 1982; Kramarsky-Winter et al. 1997; Larsson et al. 2014). Adding to this hypothesis, polyp bail-out was proposed as a potential tool for the invasion of *Tubastraea coccinea*, a highly competitive Atlantic coral, into Brazilian waters (Capel et al. 2014). However, to date, only a few reports have actually observed resettlement and re-calcification of coral polyps after bail-out (Sammarco 1982; Kvitt et al. 2015; Shapiro et al. 2016; Liu et al. 2020). On the other hand, several studies have reported viability of bailed-out coral polyps in a free-living, non-calcifying form, with intact gross morphology and possible feeding capability (Goreau and Goreau 1959; Serrano et al. 2018; Pang et al. 2020). Under low pH conditions (pH 7.2 – 7.6), Fine and Tchernov (2007) and Kvitt et al. (2015) reported induction of polyp bail-out in *Pocillopora damicornis* and a similar response (colony dissociation, but no polyp detachment) in *Oculina patagonica* and *Madracis pharencis*, which were reversible after a return to ambient pH. Moreover, accompanying decalcification, solitary polyps of *O. patagonica* showed higher polyp growth rates at lower pH compared with control colonies at ambient pH (Fine and Tchernov 2007). These observations suggest that polyp bail-out, by reallocating energy from colony development and calcification to more fundamental biological functions, may represent a means for scleractinian corals to survive unfavorable environmental conditions. More fruitful discussion of ecological roles of polyp bail-out, however, necessitates more detailed investigation of this stress response.

Polyp bail-out provides a new platform for coral research

Based on laboratory experiments, induction of polyp bail-out has been demonstrated in several coral species and by various methods (Domart-Coulon et al. 2004; Kvitt et al. 2015; Shapiro et al. 2016; Fordyce et al. 2017; Wecker et al. 2018). In addition to low pH treatments mentioned above, hyperthermal treatments induce polyp bail-out or a similar polyp-detaching response in *P. damicornis* and the cushion coral, *Cladocora caespitosa* (Kruzic 2007; Fordyce et al. 2017). In *Astroides calycularis*, an azooxanthellate coral, starvation conditions induced polyp-bail-out (Serrano et al. 2018). By manipulating seawater chemistry in the cultivation environment, such as calcium concentration or salinity, Domart-Coulon et al. (2004) and Shapiro et al. (2016) independently demonstrated induction of polyp bail-out in several pocilloporid corals (*P. damicornis*, *S. hystrix*, and *Stylophora pistillata*). By applying microfluidic techniques, more recent studies further showed that bailed-out polyps can be cultivated in the solitary morphotype under laboratory conditions for weeks or longer (Shapiro et al. 2016; Luo et al. 2020; Pang et al. 2020). These pioneering studies revealed a new model for coral research and provided an opportunity to study coral coloniality on the basis of a single coral species.

Research Aims

In this thesis, I first explore genetic mechanisms of hyperosmosis-induced polyp bail-out in *Pocillopora acuta*, a common branching coral around Okinawa, Japan. To justify application of bailed-out polyps as a coral research model, I examine health dynamics of *P. acuta* polyps after induced bail-out. Finally, using bailed-out *P. acuta* polyps as representatives of a solitary lifestyle and normal colonies as representatives of a colonial lifestyle, I explore the molecular basis of coral coloniality. Experiments in this thesis are defined in three stages, with each elaborated as a separate chapter (Figure 1).

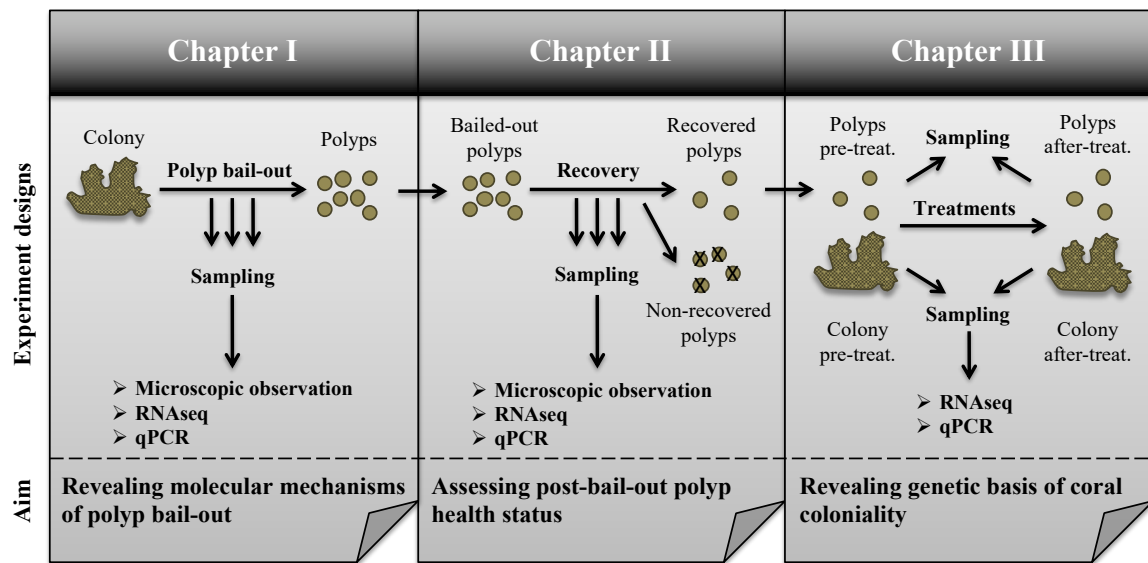


Figure 1. Experimental design and objectives of this thesis. Results of each chapter are elaborated separately.

Molecular mechanism of polyp bail-out

In Chapter 1, I demonstrate induction of polyp bail-out in *P. acuta* using controlled hyperosmotic treatments, modified from the method of Shapiro et al. (2016). Based on RNA-seq data representing multiple time points during bail-out induction, I present a *P. acuta* transcriptome assembly (GenBank accession: GIDI000000000), which allows for examination of genetic changes occurring during polyp bail-out at a whole-transcriptome level. Involved cellular processes and signaling pathways in hyperosmosis-induced bail-out are then discussed.

Recovery of coral polyps after bail-out

In Chapter 2, I present morphological and genetic dynamics of *P. acuta* polyps following hyperosmosis-induced bail-out. Based on derived data, I discuss resumption of

fundamental cellular functions in bailed-out *P. acuta* polyps. Finally, I discuss applicability of bailed-out polyps as a new model for coral research and possible ecological consequences of polyp bail-out for coral reef communities.

Genetics in coral coloniality

In Chapter 3, I apply bailed-out *P. acuta* polyps to a study of coral coloniality. Based on more comprehensive RNA-seq data of *P. acuta*, I present a more representative *P. acuta* transcriptome assembly (GenBank accession: GJAW000000000). Using this beta version of the *P. acuta* transcriptome assembly, I explore transcriptomes of normal coral colonies and bailed-out solitary polyps. Results of genetic analysis are discussed along with known morphological and physiological features at different biological scales, in order to discover differences in biological properties of solitary polyps and those in a colonial form.

1. Chapter 1

Molecular mechanism of polyp bail-out

(Published in *Coral Reefs* as “*Signaling pathways in the coral polyp bail-out response*”)

1.1 Introduction

Polyp bail-out is a coral stress response that shows potential as a coral micropropagation protocol. Zooxanthellate, solitary polyps generated from bail-out offer a model to study fundamental questions in corals, such as maintenance of photosymbiosis or coloniality. However, even though many studies have demonstrated induction of polyp bail-out under various laboratory conditions, our understanding of the molecular basis of this coral response is still in its infancy. In this chapter, I first establish a controllable hyperosmosis-based bail-out-induction protocol with *P. acuta*, modified from Shapiro et al. (2016). From RNA-seq data of *P. acuta* collected at various time points during bail-out induction, I then construct a *P. acuta* transcriptome assembly (GenBank accession: GIDI000000000) to reveal molecular mechanisms underlying polyp bail-out.

1.2 Published Article

Chuang, Po-Shun, and Satoshi Mitarai. "Signaling pathways in the coral polyp bail-out response." *Coral Reefs* 39.6 (2020): 1535-1548.

1.3 Conclusions

In this chapter, I presented an experimental protocol to induce polyp bail-out in *P. acuta*. Using this approach, polyp bail-out can be induced in a predictable manner, allowing more precise genetic analysis. By exploiting a transcriptomic analysis and qPCR techniques, I identified expression patterns of genes related to TNF signaling and apoptosis, as well as those related to FGF signaling and proteolysis during initiation of polyp bail-out. These findings reveal parallel pathways for colony dissociation and polyp detachment in polyp bail-out. Furthermore, several genes with anti-apoptotic or survival-related functions were upregulated during bail-out induction, indicating “programmed survival” of coral polyps after bail-out and supporting the escape role of this stress response. According to these findings, I hypothesized a signaling network and molecular mechanisms responsible for polyp bail-out. These findings expand our knowledge of this

coral stress response. More importantly, the polyp bail-out protocol and the *P. acuta* transcriptome assembly reported in this chapter provide a basis for examining physiology of coral polyps post-bail-out, which will be discussed in Chapter 2.

2. Chapter 2

Recovery of coral polyps after bail-out

(Published in *Frontiers in Marine Science* as “*Morphological and Genetic Recovery of Coral Polyps After Bail-Out*”)

2.1 Introduction

In recent years, several studies have successfully combined polyp bail-out and microfluidic techniques, a novel coral research platform termed “polyp-on-a-chip” (Shapiro et al. 2016; Luo et al. 2020; Pang et al. 2020). In most early studies, health status of bailed-out polyps was determined based upon survivorship or rate of resettlement. However, coral polyps have reportedly shown viability in a free-living form after bail-out, without re-attachment to substrates. Assessment of polyp health after bail-out therefore requires other analytical methods. In this chapter, I monitor morphological dynamics of *P. acuta* polyps after bail-out, induced using the protocol reported in the previous chapter. Meanwhile, using the *P. acuta* transcriptome assembly reported in Chapter 1, I examine genetic changes in bailed-out polyps of different morphologies and discuss physiological recovery of *P. acuta* polyps after the induced bail-out response.

2.2 Published Article

Chuang, Po-Shun, Kota Ishikawa, and Satoshi Mitarai. "Morphological and Genetic Recovery of Coral Polyps After Bail-Out." *Frontiers in Marine Science* 8 (2021): 280.

2.3 Conclusions

In this chapter, I demonstrated physiological recovery of *P. acuta* polyps after hyperosmosis-induced bail-out. Within five days after bail-out, I observed regeneration of morphological features, such as tentacles and body polarity, in about half of bailed-out polyps. In morphologically recovered polyps, expression recovery was found in 87% of genes altered during bail-out induction, with overrepresentation of fundamental cellular functions, including cell proliferation and metabolism. These results support applicability of bailed-out polyps to coral research and provide a basis for Chapter 3. In addition, the

sinking nature of bailed-out polyps and consistent overexpression of $\alpha 4$ integrin observed in this chapter shed light on discussions of the ecological role of polyp bail-out.

3. Chapter 3

Genetics in coral coloniality

(Published in *BMC Genomics* as “*Genetic changes involving the coral gastrovascular system support the transition between colonies and bailed-out polyps: evidence from a Pocillopora acuta transcriptome*”)

3.1 Introduction

Colonial lifestyles have appeared various times during evolution of scleractinian corals (Campoy et al. 2020). In comparison to a solitary lifestyle, colony formation enables direct communication between integrated polyps and is thought to facilitate coral responses to environmental stimuli (Swain et al. 2018). However, the molecular foundation of coloniality is still poorly understood. In Chapter 2, I showed that *P. acuta* polyps recover after an induced bail-out response, both in terms of morphology and fundamental cellular processes. These findings pave the way to study biology of coloniality using bailed-out polyps. In the final chapter of this thesis, I first assemble a new *P. acuta* transcriptome (GenBank accession: GJAW000000000). Based on this new version of the *P. acuta* transcriptome assembly, I then examine transcriptional profiles of bailed-out polyps and of normal colonies of *P. acuta* under various environmental conditions, in an effort to explore coral coloniality from a molecular perspective.

3.2 Published article

Chuang, Po-Shun, and Satoshi Mitarai. "Genetic changes involving the coral gastrovascular system support the transition between colonies and bailed-out polyps: evidence from a *Pocillopora acuta* transcriptome." *BMC genomics* 22.1 (2021): 1-12.

3.3 Conclusions

In this chapter, I revealed the genetic background underlying formation of colonies and functional modulation of the gastrovascular system in *P. acuta*. Overexpression of ACE and ECE in colonial polyps compared with bailed-out solitary polyps suggests possible participation of these enzymes in colony development in *P. acuta*, warranting functional

characterization of these genes in corals. The transition from bailed-out polyps to colonies, suggested by the genetic data in this chapter, results in different responses to environmental changes, which presumably enhance coral fitness. These findings provide additional evidence for the selective advantages of coral coloniality. Future studies with more coral representatives and other aspects of coloniality, such as budding and fragmentation, should provide further insights into the biology of coloniality in stony corals.

Conclusions

In this thesis, I explored the molecular basis of coloniality in stony corals using bailed-out polyps. I first demonstrated a protocol to induce polyp bail-out in *P. acuta*, using a controlled hyperosmotic treatment (Chapter 1). This protocol induces bail-out in a predictable and repeatable manner, enabling investigation of polyp bail-out with more precise experimental design. Combining this protocol with genetic analyses, I identified parallel signaling pathways responsible for colony dissociation, polyp detachment, and survival of polyps in the bail-out response. Following these findings, I further demonstrated recovery of coral polyps generated using the bail-out induction protocol (Chapter 2). Finally, I used bailed-out polyps as representatives of a solitary lifestyle to explore the biology of coral coloniality (Chapter 3). Based on transcriptomic data, I proposed emergence of neurological and circulatory system development, and possible participation of angiotensin-converting enzymes and endothelin-converting enzymes during colony formation, which lead to different stress responses. These findings expand our knowledge of coloniality in stony corals. However, differences between colonies and bailed-out polyps identified in this thesis should not be interpreted as undermining the applicability of solitary polyps generated from polyp bail-out. Rather, the findings in this thesis pave the way for future applications of polyp bail-out and solitary polyps derived from it, which will prove useful in studying more fundamental questions in corals, such as characterization of genes or cellular processes of interest.

Bibliography

- Barbeitos MS, Romano SL, Lasker HR (2010) Repeated loss of coloniality and symbiosis in scleractinian corals. *Proceedings of the National Academy of Sciences* 107:11877-11882
- Bates NR (2002) Seasonal variability of the effect of coral reefs on seawater CO₂ and air—sea CO₂ exchange. *Limnology and Oceanography* 47:43-52
- Bo J, Yao J, Liao W, Deng Z (2017) Triassic scleractinian corals in China: A review of present knowledge. *Acta Geologica Sinica-English Edition* 91:270-282
- Brown B (1997) Coral bleaching: causes and consequences. *Coral reefs* 16:S129-S138
- Campoy AN, Addamo AM, Machordom A, Meade A, Rivadeneira MM, Hernández CE, Venditti C (2020) The origin and correlated evolution of symbiosis and coloniality in scleractinian corals. *Frontiers in Marine Science* 7:461
- Capel KCC, Migotto A, Zilberberg C, Kitahara MV (2014) Another tool towards invasion? Polyp “bail-out” in *Tubastraea coccinea*. *Coral Reefs* 33:1165-1165
- Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortés J, Delbeek JC, DeVantier L (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* 321:560-563
- Cesar H, Burke L, Pet-Soede L (2003) The economics of worldwide coral reef degradation
- Cesar HS (2000) Coral reefs: their functions, threats and economic value. *Collected essays on the economics of coral reefs* 14
- Chisholm JR, Barnes DJ (1998) Anomalies in coral reef community metabolism and their potential importance in the reef CO₂ source-sink debate. *Proceedings of the National Academy of Sciences* 95:6566-6569
- Chuang P-S, Mitarai S (2020) Signaling pathways in the coral polyp bail-out response. *Coral Reefs*:1-14
- Chuang P-S, Mitarai S (2021) Genetic changes involving the coral gastrovascular system support the transition between colonies and bailed-out polyps: evidence from a *Pocillopora acuta* transcriptome. *BMC genomics* 22:1-12
- Chuang P-S, Ishikawa K, Mitarai S (2021) Morphological and Genetic Recovery of Coral Polyps After Bail-Out. *Frontiers in Marine Science* 8:280
- Cohen I, Dubinsky Z, Erez J (2016) Light enhanced calcification in hermatypic corals: new insights from light spectral responses. *Frontiers in Marine Science* 2:122
- Crossland C, Hatcher B, Smith S (1991) Role of coral reefs in global ocean production. *Coral reefs* 10:55-64
- Domart-Coulon I, Tambutté S, Tambutté E, Allemand D (2004) Short term viability of soft tissue detached from the skeleton of reef-building corals. *Journal of experimental marine biology and ecology* 309:199-217
- Douglas A (2003) Coral bleaching—how and why? *Marine pollution bulletin* 46:385-392
- Fine M, Tchernov D (2007) Scleractinian coral species survive and recover from decalcification. *Science* 315:1811-1811

- Fine M, Oren U, Loya Y (2002) Bleaching effect on regeneration and resource translocation in the coral *Oculina patagonica*. *Marine Ecology Progress Series* 234:119-125
- Fordyce AJ, Camp EF, Ainsworth TD (2017) Polyp bailout in *Pocillopora damicornis* following thermal stress. *F1000Research* 6
- Gateno D, Israel A, Barki Y, Rinkevich B (1998) Gastrovascular circulation in an octocoral: evidence of significant transport of coral and symbiont cells. *The Biological Bulletin* 194:178-186
- Gattuso J-P, Frankignoulle M, Smith SV (1999) Measurement of community metabolism and significance in the coral reef CO₂ source-sink debate. *Proceedings of the National Academy of Sciences* 96:13017-13022
- Geider RJ, Delucia EH, Falkowski PG, Finzi AC, Grime JP, Grace J, Kana TM, La Roche J, Long SP, Osborne BA (2001) Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Global change biology* 7:849-882
- Gladfelter EH (1983) Circulation of fluids in the gastrovascular system of the reef coral *Acropora cervicornis*. *The Biological Bulletin* 165:619-636
- Goreau TF, Goreau NI (1959) The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. *The Biological Bulletin* 117:239-250
- Hatcher BG (1988) Coral reef primary productivity: a beggar's banquet. *Trends in Ecology & Evolution* 3:106-111
- Hiebert LS, Simpson C, Tiozzo S (2020) Coloniality, clonality, and modularity in animals: The elephant in the room. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*
- Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and freshwater research* 50:839-866
- Kawahata H, Suzuki A, Goto K (1997) Coral reef ecosystems as a source of atmospheric CO₂: evidence from pCO₂ measurements of surface waters. *Coral Reefs* 16:261-266
- Kinsey D, Hopley D (1991) The significance of coral reefs as global carbon sinks—response to greenhouse. *Palaeogeography, Palaeoclimatology, Palaeoecology* 89:363-377
- Knowlton N, Brainard RE, Fisher R, Moews M, Plaisance L, Caley MJ (2010) Coral reef biodiversity. *Life in the world's oceans: diversity distribution and abundance*:65-74
- Kramarsky-Winter E, Fine M, Loya Y (1997) Coral polyp expulsion. *Nature* 387:137-137
- Kruzic P (2007) Polyp expulsion of the coral *Cladocora caespitosa* (Anthozoa, Scleractinia) in extreme sea temperature conditions. *Natura Croatica* 16:211
- Kvitt H, Kramarsky-Winter E, Maor-Landaw K, Zandbank K, Kushmaro A, Rosenfeld H, Fine M, Tchernov D (2015) Breakdown of coral colonial form under reduced pH conditions is initiated in polyps and mediated through apoptosis. *Proceedings of the National Academy of Sciences* 112:2082-2086

- Larsson AI, Järnegren J, Strömberg SM, Dahl MP, Lundälv T, Brooke S (2014) Embryogenesis and larval biology of the cold-water coral *Lophelia pertusa*. *PLoS One* 9:e102222
- Liu C, Cheng SH, Lin S (2020) Illuminating the dark depths inside coral. *Cellular Microbiology* 22:e13122
- Luo Y, Zhao J, He C, Lu Z, Lu X (2020) Miniaturized Platform for Individual Coral Polyps Culture and Monitoring. *Micromachines* 11:127
- McFarlane I, Callan HG (1978) Multiple conducting systems and the control of behaviour in the brain coral *Meandrina meandrites* (L.). *Proceedings of the Royal Society of London Series B Biological Sciences* 200:193-216
- Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. *Ecological economics* 29:215-233
- Moya A, Tambutté S, Tambutté E, Zoccola D, Caminiti N, Allemand D (2006) Study of calcification during a daily cycle of the coral *Stylophora pistillata*: implications for light-enhanced calcification'. *Journal of Experimental Biology* 209:3413-3419
- Munro JL (1996) The scope of tropical reef fisheries and their management Reef fisheries. Springer, pp1-14
- Muscantine L, Goiran C, Land L, Jaubert J, Cuif J-P, Allemand D (2005) Stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of organic matrix from coral skeleton. *Proceedings of the National Academy of Sciences* 102:1525-1530
- Pac S (1978) Coral-reef area and the contributions of reefs to processes and resources of the world's oceans. *Nature* 273:18
- Pang A-P, Luo Y, He C, Lu Z, Lu X (2020) A polyp-on-chip for coral long-term culture. *Scientific Reports* 10:1-9
- Reaka-Kudla ML (1997) The global biodiversity of coral reefs: a comparison with rain forests. *Biodiversity II: Understanding and protecting our biological resources* 2:551
- Rees SA, Opdyke BN, Wilson PA, Fifield LK (2005) Coral reef sedimentation on Rodrigues and the Western Indian Ocean and its impact on the carbon cycle. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 363:101-120
- Roberts CM, McClean CJ, Veron JE, Hawkins JP, Allen GR, McAllister DE, Mittermeier CG, Schueler FW, Spalding M, Wells F (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science* 295:1280-1284
- Roff G, Hoegh-Guldberg O, Fine M (2006) Intra-colonial response to Acroporid “white syndrome” lesions in tabular *Acropora* spp.(Scleractinia). *Coral Reefs* 25:255-264
- Romano S, Cairns SD (2000) Molecular phylogenetic hypotheses for the evolution of scleractinian corals. *Bulletin of Marine Science* 67:1043-1068
- Romano SL, Palumbi SR (1996) Evolution of scleractinian corals inferred from molecular systematics. *Science* 271:640-642
- Sammarco PW (1982) Polyp bail-out: an escape response to environmental stress and a new means of reproduction in corals. *Marine ecology progress series* Oldendorf 10:57-65

- Sargent MC, Austin TS (1949) Organic productivity of an atoll. *Eos, Transactions American Geophysical Union* 30:245-249
- Serrano E, Coma R, Inostroza K, Serrano O (2018) Polyp bail-out by the coral *Astroides calycularis* (Scleractinia, Dendrophylliidae). *Marine Biodiversity* 48:1661-1665
- Shapiro OH, Kramarsky-Winter E, Gavish AR, Stocker R, Vardi A (2016) A coral-on-a-chip microfluidic platform enabling live-imaging microscopy of reef-building corals. *Nature communications* 7:10860
- Simpson C (2013) Species selection and the macroevolution of coral coloniality and photosymbiosis. *Evolution* 67:1607-1621
- Spalding M, Grenfell A (1997) New estimates of global and regional coral reef areas. *Coral reefs* 16:225-230
- Spalding M, Spalding MD, Ravilious C, Green EP (2001) *World atlas of coral reefs*. Univ of California Press
- Spurgeon JP (1992) The economic valuation of coral reefs. *Marine pollution bulletin* 24:529-536
- Stanley GD (1988) The history of early Mesozoic reef communities; a three-step process. *Palaios* 3:170-183
- Stanley GD, Fautin DG (2001) The origins of modern corals. *Science* 291:1913-1914
- Stanley Jr GD (2003) The evolution of modern corals and their early history. *Earth-Science Reviews* 60:195-225
- Stanley Jr GD, Helmle KP (2010) Middle Triassic coral growth bands and their implication for photosymbiosis. *Palaios* 25:754-763
- Stolarski J, Kitahara MV, Miller DJ, Cairns SD, Mazur M, Meibom A (2011) The ancient evolutionary origins of Scleractinia revealed by azooxanthellate corals. *BMC evolutionary biology* 11:1-11
- Swain TD, Bold EC, Osborn PC, Baird AH, Westneat MW, Backman V, Marcelino LA (2018) Physiological integration of coral colonies is correlated with bleaching resistance. *Marine Ecology Progress Series* 586:1-10
- Ware JR, Smith SV, Reaka-Kudla ML (1992) Coral reefs: sources or sinks of atmospheric CO₂? *Coral reefs* 11:127-130
- Wecker P, Lecellier G, Guibert I, Zhou Y, Bonnard I, Berteaux-Lecellier V (2018) Exposure to the environmentally-persistent insecticide chlordecone induces detoxification genes and causes polyp bail-out in the coral *P. damicornis*. *Chemosphere* 195:190-200
- Zhanqiu D, Lei K (1984) Middle Triassic corals and sponges from southern Guizhou and eastern Yunnan. *Acta Palaeontologica Sinica* 23:490-503