Title
Ion-regulatory and developmental physiology of giant clams (Genus Tridacna) and their conservation status on the island of Mo’orea, French Polynesia.

Permalink
https://escholarship.org/uc/item/74f4p4jq

Author
Armstrong, Eric James

Publication Date
2017

Peer reviewed|Thesis/dissertation
Ion-regulatory and developmental physiology of giant clams (Genus *Tridacna*) and their conservation status on the island of Mo’orea, French Polynesia.

By

Eric J. Armstrong

A dissertation submitted in partial satisfaction of the requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Jonathon H. Stillman, Co-Chair
Professor Mary E. Power, Co-Chair
Professor Caroline M. Williams
Professor Rosemary G. Gillespie

Fall 2017
Ion-regulatory and developmental physiology of giant clams (Genus *Tridacna*) and their conservation status on the island of Mo’orea, French Polynesia.

© 2017

by

Eric J. Armstrong
Abstract

Ion-regulatory and developmental physiology of giant clams (Genus *Tridacna*) and their conservation status on the island of Mo’orea, French Polynesia.

By

Eric J. Armstrong

Doctor of Philosophy in Integrative Biology
University of California, Berkeley

Professor Jonathon H. Stillman, Co-Chair
Professor Mary E. Power, Co-Chair

Marine species face several challenges as a result of human activities including direct effects on populations (e.g. overharvesting) and indirect impacts on individuals (e.g. physiological responses to ocean warming and acidification). Ameliorating these impacts requires successful regulation at both the population and physiological levels. One group of critically threatened organisms are the so called “giant clams” (genus *Tridacna*) of tropical coral reefs. Like corals, giant clams possess calcifying larvae and host symbiotic microalgae and are therefore susceptible to environmentally-driven failure of biomineralization and symbiotic disruption. In addition, giant clams are economically important fisheries species which have been severely overexploited throughout much of their range. While marine reserves have been established to replenish dwindling giant clam stocks, their efficacy in promoting population recovery remains unknown. Similarly, little is known regarding the magnitude of climate-change associated physiological effects on giant clams. This stems, in part, from a lack of knowledge regarding the response of early life-history stages to warming and acidification and because the molecular mechanisms regulating acid-base homeostasis and symbiont photosynthesis in giant clams remain poorly characterized. I addressed these knowledge gaps in populations of the small giant clam, *Tridacna maxima*, from across a network of marine protected areas (MPAs) on Mo’orea, French Polynesia. I employed a combined physiological and ecological approach to (1) investigate mechanistic processes underlying acid-base regulation within giant clams, specifically in relation to maintenance of host-symbiont homeostasis, (2) measure the effects of increased temperature and elevated $pCO_2$ on giant clam fertilization success, and (3) assess the efficacy of a recently established Marine Protected Area Network in promoting conservation and recovery of this species in an exploitative environment.

I demonstrate that giant clams regulate symbiont photosynthesis through the activity of an ion-transport protein, vacuolar-type H$^+$-ATPase (VHA), which is strongly localized in close proximity to symbiotic algae. I further show that clam VHA actively promotes algal photosynthesis, increasing rates of O$_2$ production and holobiont metabolic rate, and likely represents a convergent exaptation for carbon concentration shared by reef-building corals. This process has implications for climate-related responses and may offset the negative impacts of future ocean acidification in these species. I also present the first data demonstrating giant clam
early life-history responses to climate change drivers and show that syngamy in giant clams is extremely sensitive to environmental warming. Finally, I demonstrate the significant, positive, effect of MPA establishment in permitting recovery of overharvested giant clam populations. *T. maxima* populations have increased approximately 3-fold in Mo’orea’s protected sites relative to non-protected controls and this rate of recovery is significantly higher than the global average for marine reserves. Taken together, these results suggest that effective regulation at both the population and physiological level may permit the recovery and persistence of giant clams in the face of anthropogenic challenges.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>viii</td>
</tr>
<tr>
<td><strong>CHAPTER 1</strong></td>
<td>1</td>
</tr>
<tr>
<td>Symbiont photosynthesis in giant clams is promoted by host H+ -pumping.</td>
<td></td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS</td>
<td>3</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>6</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>9</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>9</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>10</td>
</tr>
<tr>
<td>FIGURE CAPTIONS</td>
<td>14</td>
</tr>
<tr>
<td>FIGURES</td>
<td>15</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>20</td>
</tr>
<tr>
<td><strong>CHAPTER 2</strong></td>
<td>24</td>
</tr>
<tr>
<td>Elevated temperature, but not acidification, reduces fertilization success in the small giant clam, Tridacna maxima.</td>
<td></td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>24</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>24</td>
</tr>
<tr>
<td>METHODS</td>
<td>26</td>
</tr>
<tr>
<td>RESULTS</td>
<td>28</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>29</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>31</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>31</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>32</td>
</tr>
<tr>
<td>TABLES</td>
<td>37</td>
</tr>
<tr>
<td>FIGURE CAPTIONS</td>
<td>39</td>
</tr>
<tr>
<td>FIGURES</td>
<td>40</td>
</tr>
</tbody>
</table>
CHAPTER 3

Symbiont photosynthesis in giant clams is promoted Marine reserves have facilitated rapid recovery of Tridacna maxima on the island of Mo’orea, French Polynesia.

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>43</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>43</td>
</tr>
<tr>
<td>METHODS</td>
<td>46</td>
</tr>
<tr>
<td>RESULTS</td>
<td>47</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>48</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>50</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>52</td>
</tr>
<tr>
<td>TABLES</td>
<td>57</td>
</tr>
<tr>
<td>FIGURE CAPTIONS</td>
<td>58</td>
</tr>
<tr>
<td>FIGURES</td>
<td>59</td>
</tr>
</tbody>
</table>
INTRODUCTION

The world’s oceans are entering an era of profound change, with rapid and major deviations from the abiotic conditions which have prevailed largely unaltered since the start of the Neogene circa 25 mya (Jackson 2008). Driven mainly by anthropogenic emissions of carbon dioxide (CO₂), this rapid change has resulted in a thermal destabilization of Earth’s climate system with global mean temperature expected to rise by as much as +4 °C over the next century (IPCC 2007). The biological consequences of this “global warming” are already significant (Stillman and Armstrong 2015) and are likely to continue given the importance of temperature as a biological forcing agent. Indeed, it has long been argued that temperature is the single most important driver affecting biological systems influencing as it does every aspect of an organism’s physiology from the behavior of molecules to the integration of complex systems within the whole organism (Hochachka and Somero 2001). In recent years, increased mean annual temperatures have been shown to have significant consequences for many marine taxa including large-scale range shifts (Sorte et al. 2010), and an increase in the frequency and severity of “bleaching events” which are disruptions in animal-algal symbioses (Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2007; Logan et al. 2014). However, climate responses to increased atmospheric CO₂ are diverse and include alterations in many other environmental variables than temperature alone.

An additional example is the large-scale accumulation of CO₂ in the oceans which has resulted in a reduction of oceanic pH commonly known as ocean acidification. Reduced pH and accompanying reductions in the saturation state of the exoskeletal mineral aragonite have been experimentally shown to negatively impact fertilization success and to drastically reduce larval survival in many taxa (Kurihara 2008; Byrne 2011a; Byrne and Przeslawski 2013). In combination, these climatic trends in temperature and pH threaten the diversity and stability of marine ecosystems across the globe and may have especially strong impacts in the tropics where many species may already be living at or near their respective thermal maxima and have limited acclimation capacity (Stillman 2003). Within such ecosystems, the most vulnerable organisms are likely to be calcifying marine invertebrates which cannot decouple changes in environmental temperature from alterations in body temperature (i.e. are poikilothermic) and whose carbonate shells are prone to dissolution under the synergistic effects of elevated temperature and pCO₂ (Toonen et al. 2011).

In addition to these abiotic challenges, marine populations face the direct threat of overexploitation as a result of commercial and local harvesting efforts (Aburto-Oropeza et al. 2011). Recent analyses indicate that as much as 63% of assessed fish stocks worldwide have been overharvested and require rebuilding, and suggest that the establishment of lower exploitation rates are needed to prevent the collapse of currently vulnerable species (Worm et al. 2009). No-take marine reserves have been promoted as effective tools for protecting vulnerable species, promoting biodiversity, maintaining ecosystem function, and permitting recovery of overexploited species (Balmford et al. 2004; Aburto-Oropeza et al. 2011). However, less than 0.1% of the world’s oceans are currently protected from extractive activities and increasing coastal populations are putting further strain on even the most remote marine resources (Vieux et al. 2004).
At the intersection of all three of these challenges (warming, acidification, and overexploitation) lie coral reef ecosystems. Coral reef ecosystems are some of the most diverse habitats on earth and are of major importance to many island communities where they support the development of local and national economies (Ferraris and Cayré 2003; Kronen et al. 2010; Pickering et al. 2011). Reefs, however, are becoming critically threatened by overfishing as a result of overpopulation and overexploitation of commercial fisheries species (Hughes 2003; Pandolfi et al. 2003). A recent global analysis has indicated that 75% of coral reef ecosystems are currently severely threatened by anthropogenic pressure with this figure is predicted to rise to 90% by 2030 and 100% by 2050 (Burke et al. 2011). Even in remote regions of the South Pacific where human impacts on reefs have been less intense (Vieux et al. 2004), extractive local economies have resulted in incipient overexploitation of marine resources (Planes et al. 1993; Van Wynnberge et al. 2016). Within coral reef ecosystems, the most vulnerable organisms are likely to be corals themselves along with other calcifying marine invertebrates like giant clams (genus Tridacna) which cannot decouple changes in environmental temperature from alterations in body temperature (i.e. are poikilothermic) and whose carbonate shells are prone to dissolution under the synergistic effects of elevated temperature and $pCO_2$. Among those species of special concern are the so-called “giant clams” of the genus *Tridacna*.

Giant clams are the largest living bivalves and are found throughout the tropical Indo-Pacific where they rank among the most charismatic of coral reef inhabitants. Though tridacnid clams share characteristics with other well-studied bivalves, they are unique in their ability to host symbiotic dinoflagellate algae, genus Symbiodinium, and are important both culturally and economically in many developing island territories (e.g. the Solomon Islands) where they are harvested for the seafood, ornamental shell and aquarium hobbyist trades (Pickering et al. 2011; Toonen et al. 2011). In these regions, giant clam products can rank as the number one national export with annual export production values exceeding $90 million USD or ~5% of total Gross Domestic Product (Solomon Islands, 2011 UN World Statistics GDP estimates). As a result of these intensive harvesting efforts, many wild stocks of giant clams are now heavily overexploited and all species are currently listed under the Convention on International Trade in Endangered Species (CITES) as endangered species of concern, vulnerable to local extirpations (Lucas et al. 1989; Firdausy and Tisdell 1992; Neo and Todd 2013).

Although commercial efforts are currently underway to utilize mariculture of *T. maxima* to meet market demands and replenish wild stocks, these efforts are likely to be hampered by a lack of knowledge regarding *T. maxima*’s vulnerability to ocean warming and acidification predicted to result from climate change (Watson et al. 2012). Unlike many other important mariculture species, few studies have explicitly investigated the effects of climate drivers on giant clam physiology and none have investigated these effects in larval stages. Juvenile *Tridacna squamosa* giant clams suffered 81% lower survivorship after 60 days of exposure to ocean conditions predicted by the year 2100 (Watson et al. 2012). However, these negative effects could be offset by increased symbiont photosynthesis (Watson 2015) suggesting that, through the activity of its algal partners, the *Tridacna*Symbiodinium holobiont may be resilient to ocean acidification. Despite the clear and encouraging implications of these findings for management of this species under future ocean conditions, the physiological mechanisms behind holobiont photosynthetic regulation remain unresolved. Furthermore, our lack of understanding of the effects of ocean warming and
acidification on early life history stages severely limits our ability to make predictions about how giant clams species may fare under a changing climate.

In this thesis, I address these knowledge gaps using a combined physiological and ecological approach in the small giant clam, *Tridacna maxima*. In Chapter 1, I discuss the role that a class of ion-transport proteins, the vacuolar H+-ATPases (VHAs), play in regulating carbon flow between giant clams and their dinoflagellate symbionts. I also discuss potential implications of this carbon concentration mechanism for persistence of giant clams under a changing climate. In Chapter 2, I investigate the response of fertilization in giant clams to exposure to elevated temperature and $pCO_2$ congruent with predictions of ocean conditions by the end of the century. My data show, that while adult giant clams may be resilient to the effects of ocean warming and acidification, early life history stages are likely extremely vulnerable to elevated temperature. In Chapter 3, I assess the efficacy of a series of marine protected areas, the Plan de Gestion de l’Espace Maritime network (PGEM), established in 2004 on the island of Mo’orea, French Polynesia, for preserving and promoting recovery of previously overexploited populations of the small giant clam, *Tridacna maxima*. I document the phenomenal success these protected areas have had in restoring populations of giant clams on Mo’orea’s reefs and discuss the potential role of local involvement and Polynesian cultural practices in effecting this success. I envision that the paired physiological and transcriptomic approach of this study will serve as a comprehensive system for elucidating the molecular mechanisms underlying this giant clam species’ physiological responses to ocean warming and acidification – information which is vitally relevant for future management of these important threatened species under future ocean conditions.

**REFERENCES**


Byrne M (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: Vulnerabilities and potential for persistence in a changing ocean. 1–42.


Pickering TD, Ponia B, Hair CA, Southgate, P.C. Poloczanska, E.S., Patrona LD, Teitelbaum A,
Mohan CV, Phillips MJ, Bell JD, Silva SD (2011) Vulnerability of Tropical Pacific Fisheries and Aquaculture to Climate Change. Secretariat of the Pacific Community, Noumea, New Caledonia


ACKNOWLEDGEMENTS

“It is good to have an end to journey toward, but it is the journey that matters in the end.”
— Ursula K. Le Guin

The completion of this thesis has been one of the most humbling and rewarding journeys of my life. Along the way I’ve been blessed with the help and support of many colleagues, friends, and family whose intellect, comradery, and humor made this undertaking at some times more bearable, and at all times more enjoyable. Any summary such as this is bound to be incomplete, but to all those friends, mentioned and unmentioned, who have been a part this endeavor I say "thank you", deeply and from the heart. Without the support and inspiration of these, and many other, truly incredible and brilliant people I’m come to know throughout this endeavor, this work would never have been completed.

First and foremost, I am grateful for the support and mentorship provided over the past six years by my advisor, Jonathon Stillman. His passion and expertise in designing truly integrative projects have made me a better scientist and, I’m eternally grateful for his encouragement to develop an independent project on the physiology and natural history of giant clams. I’m also exceedingly grateful for the many professional development opportunities he has opened-up for me including numerous introductions over the years to leaders in the field of ecophysiology, some of whom I’m happy now to know as friends. I would also like to thank my committee members Mary Power, Caroline Williams, and Rosie Gillespie for their guidance and support. All three are among the most brilliant people I’ve had the privilege of interacting with. As my co-advisor, Mary Power took a chance on me as a student and I’m eternally grateful for that opportunity. From our very first conversation about Robin Wall Kimmerer’s beautiful book “Gathering Moss”, I’ve been awed at Mary’s capacity to so effortlessly blend rigorous science with a love and celebration of the organisms under study. I’ve also learned from her the sheer joy and power of adopting an interdisciplinary approach to research. I have immense gratitude and respect for Caroline Williams whose insightful conversations always opened my eyes to a new way of seeing approaching ecophysiological hypotheses and whose thoughtful review of my work greatly improved the quality of this manuscript. Last, but not least, a thank you to Rosemary Gillespie, for our long conversations about the potential of physiological sensitivity as an agent of isolation and who first instilled in me the wonder and magic of island ecosystems.

I am also grateful to the following colleagues and staff for their time and generosity in contributing valuable advice (academic or otherwise), feedback, and/or reagents: Alex Gunderson (UC Berkeley), Piero Calosi (U Plymouth / U Rimouski), Martin Tresguerres (Scripps Institution of Oceanography), Nathan Miller (San Francisco State University), Cindy Looy (UC Berkeley), Justin Brashares (UC Berkeley), Brent Mishler (UC Berkeley), Stephanie Carlson (UC Berkeley), David Lindberg (UC Berkeley), Vince Resh (UC Berkeley), Sue-Ann Watson (James Cook University), George Somero (Hopkins Marine Station), and Masaya Morita (Sesoko Marine Station).
I was incredibly fortunate to conduct much of this work among the beautiful islands and even more beautiful people of French Polynesia. I am eternally indebted to the faculty, staff, and students of the Richard B. Gump and CRIOBE field stations on Mo’orea, for introducing me to the thrills of tropical field research. In particular, I owe an immense debt of gratitude to Vaimiti Dubousquet who was instrumental in getting this research started. I arrived a stranger in a strange land and she welcomed me with more warmth than I could have expected or will ever be able to repay. Her scholarship and dedication are truly worthy of emulation. Mauruuru roa et de gros bisous à elle! To Maeva Beltrand, Agatha Korczak, and Isis Asset whose humor, support, and willingness to speak English with me made the endless lab hours at CRIOBE immensely more enjoyable. To Suzie Mills (CRIOBE) and Ricardo Beldade (CRIOBE) for providing me with such an incredible place to conduct my research and for their support, mentorship, and friendship which vastly ameliorated the taxing trials of sea hare mariculture. I was also incredibly fortunate to twice teach the Mo’orea Field Course at the Gump Station where my life was forever changed through my interactions with the amazingly talented students and the incredibly kind and dedicated staff. In particular, I am grateful to Valentine Brotherson and Irma You Sing for their help and their contagious positivity. To my fellow graduate student instructors, Camilla Souto, Dave Kurz, Natalie Stauffer-Olson, and Ignacio Escalante - those warm days on the beach and dark nights amongst urchin spines and mangrove roots are some of my most cherished memories from graduate school.

I owe an especially great debt of gratitude to my friends Tessa Page and Richelle Tanner who are among the most brilliant scientists I know and whose constant advice and support made this journey possible. Thanks, too, to Nick Burnett for offering me the chance (i.e. forcing me) to get out of the lab every so often and see more of the beautiful California coast – those frigid mornings searching through the surf are still some of the best. My deepest gratitude to Adam Paganini and Rachel Diner for teaching me the ropes of the Stillman Lab all those years ago and for whose infectious humor and spirit made the Romberg Tiburon Center such a great place to start my career. To my roommates, Emily Lam, Emily King, and Marty McCoy, thanks for being such amazing positive influences in my life and for daily putting up with my sarcasm. To my friends from Plymouth, Théophile Lienhardt, Leela Chakravarti, and Michael Jarrold who made the first major scientific projects of my career so much more fun to complete, a warm “4th of July” toast. There are truly no better people in the world to talk science with over a glass of cider. Finally, I’m thankful every day for my incredible good fortune in having met Bastien Bouchaud. His friendship, love, and the occasional encouraging prod to get back to writing went a long way towards seeing this work through to completion.

To my family – Arthur, Barbara, and Brittany – no words can suffice to convey my gratitude. Their unconditional love has been a constant throughout my life and their encouragement has given me the confidence to pursue my passions fearlessly. Although I never did fulfill my childhood dream of becoming a paleontologist, I didn’t land too far off-track. Everything I’ve achieved I owe to their unwavering support. I also owe them thanks for their help in completing
the work associated with Chapter 3; while there are certainly more arduous tasks than snorkeling on Tahitian reefs, I’m eternally grateful for the time spent in paradise with them.

Finally, immense thanks to Richard Hill (Michigan State University), who was the primary catalyst in my academic career and who first introduced me to the field of ecophysiology. His knowledge, passion, and dedication to science are truly inspiring. Ever since he shepherded my first field season on the sea, I’ve been grateful for the monumental, positive, difference he’s made in my life. It has been an honor, privilege, and joy going from student to friend with such a kind, generous, and brilliant mentor.

I’m also grateful to the following funding agencies for their support in completing the work presented herein: The United States Department of Defense National Defense Science and Engineering Fellowship, the UC Berkeley Graduate Division, the Berkeley Chapter of Sigma Xi, the Society for Experimental Biology, and the Company of Biologists.
CHAPTER 1  |  Symbiont photosynthesis in giant clams is promoted by host H⁺-pumping.

Eric J. Armstrong, Jinae N. Roa, Martin Tresguerres & Jonathon H. Stillman

ABSTRACT

Giant clams (genus Tridacna) are the largest living bivalves and, like reef-building corals, host symbiotic dinoflagellate algae (Symbiodinium) which are energetically important. Consequently, giant clams are highly anatomically modified to facilitate the capture of light for photosynthesis. However, in addition to light, photosynthesis requires the supply of inorganic carbon. In corals, vacuolar-type H⁺-ATPase (VHA) has been implicated as a carbon concentrating mechanism (CCM) and VHA-induced acidification of the coral symbiosome leads to increased Symbiodinium productivity. We investigated whether VHA plays an analogous role in the small giant clam, Tridacna maxima. We demonstrate that VHA is abundantly expressed in epithelial cells of Symbiodinium-containing tubules in the siphonal mantle of T. maxima, and that inhibition of VHA impairs photosynthetic oxygen production by ~40%. Given that algal productivity in light-exposed mantle increases holobiont aerobic metabolism ~five-fold and that translocated fixed carbon exceeds metabolic demand, we conclude that VHA activity in the siphonal mantle confers strong energetic benefits to the host clam potentially permitting the evolution of gigantism in the Tridacna lineage. Furthermore, the demonstrated convergent role of VHA in promoting Symbiodinium photosynthesis in giant clams and corals suggests VHA-driven CCM as a common exaptation in photosymbioses that deserves further investigation in other taxa.

INTRODUCTION

Photosymbiosis, a partnership in which a host organism harbors photosynthetic microbial or algal cells, provides competitive advantages to hosts through increased energy availability, and to symbionts through translocation of nutrients, particularly dissolved organic nitrogen (Roth 2014). Perhaps the most famous and well-studied photosymbiosis is the partnership between dinoflagellate algae of the genus Symbiodinium and their cnidarian coral hosts. However, similar symbiotic partnerships have independently arisen several times in shell-bearing molluscs, especially within the family Cardiidae where adoption of a photosymbiotic habit has evolved at least six times (Vermeij 2013) including between the “giant clams” (formerly Tridacnidae (Herrera et al. 2015)) and their algal symbionts.

Giant clams are the largest living bivalves and are found throughout the tropical Indo-Pacific, where they live in close association with reef-building corals (Knop 1996). All described tridacnid species (Othman et al. 2010; Huelsken et al. 2013), and two closely related species of the genus Hippopus, exhibit symbiotic partnerships with Symbiodinium similar to those found in reef-building corals. While corals host Symbiodinium in an intracellular compartment in...
gastrodermal cells called the symbiosome (reviewed in (Tresguerres et al. 2017)), Symbiodinium in giant clams are hosted extracellularly in modified extensions of the digestive system (termed zooxanthellae tubules). These tubes extend upwards from the stomach into the light-exposed tissue of the siphonal mantle where they are ultimately arranged roughly perpendicular to incoming solar radiation (Fig. 1A) (Norton and Jones 1992; Knop 1996; Holt et al. 2014).

Though the physical arrangement of symbionts differs between scleractinian corals and giant clams, the physiological benefits to the host are similar. Symbiont photosynthesis can provide more reduced carbon than is required to meet respiratory demand in both coral (~150%) (Muscatine et al. 1984) and Tridacna (up to 400%) (Klumpp and Griffith 1994). Furthermore, Symbiodinium photosynthesis likely enhances rates of calcification in giant clams as has been previously demonstrated in corals and several other photosymbiotic calcifiers (Vermeij 2013; Tornabene et al. 2017). However, the cellular mechanisms regulating exchange of compounds between giant clams and their partners remain largely unknown.

Regulation of symbiont physiology is essential for the success of the photosymbiosis. Unregulated supply of light, inorganic carbon or nutrients to the photosymbiont can result in algal overgrowth and strain on host resources (particularly nitrogen) (McConnaughey 2012; Wooldridge 2013). In addition, mismatches between light and CO$_2$ availability can increase the generation of damaging reactive oxygen species (Wooldridge 2013) and both corals and giant clams invest in metabolites which defend against photoinhibition and photostress (Hill et al. 2017). In extreme cases, regulatory failure can result in the expulsion of symbiotic algal partners, a process known as bleaching. Although rarely fatal in giant clams, bleaching results in significant decreases in hemolymph pH and glucose concentration and a loss in the clam’s ability to assimilate ammonium from the environment which is a crucial physiological function in the nitrogen-limited reef ecosystems giant clams inhabit (Leggat et al. 2003).

In corals, symbiont photosynthesis is strongly regulated by the cnidarian host through activity of host vacuolar-type H$^+$-ATPase (VHA) (Barott et al. 2015). VHA is an enzyme found in all eukaryotes, and utilizes energy from ATP hydrolysis to transport H$^+$ across biological membranes (Stevens and Forgac 1997; Tresguerres 2016). The translocated H$^+$ can be used to energize the transport of other molecules and, if co-transported with an anion, can mediate acid secretion (Wieczorek et al. 1999; Nelson and Harvey 1999; Tresguerres 2016). In coral, VHA acidifies the symbiosomal compartment where Symbiodinium resides, which drives speciation of inorganic carbon into CO$_2$ (Barott et al. 2015). This carbon concentrating mechanism (CCM) is essential because dinoflagellate RuBisCo, the terminal enzyme in carbon fixation, has low affinity for CO$_2$ (Rowan et al. 1996; Leggat et al. 2002). In coral, a reduction of VHA activity reduces net photosynthetic oxygen production by 30-80%, showing that VHA-induced acidification of the symbiosome promotes photosynthesis in scleractinian coral hosts (Barott et al. 2015). However, the mechanisms underlying carbon transfer between host and symbionts in giant clams remain unresolved.

In this study, we investigated whether VHA-mediated H$^+$ transport is similarly used by tridacnid clams to promote algal photosynthetic production in siphonal mantle tissue. We chose to address these questions in the small giant clam, Tridacna maxima, which has one of the largest
biogeographic ranges of any tridacnid species (Knop 1996) and has both ecological and economic importance (Neo et al. 2015; Van Wynsberge et al. 2016).

METHODS

Organism acquisition and husbandry

For metabolic rate and VHA localization experiments, juvenile T. maxima clams (n = 18; shell length 4.7 ± 0.4 cm, x ± SD) were purchased from Oceans, Reefs & Aquariums® Aquaculture company (Florida, USA) and held for acclimation in a 288 L, recirculating seawater aquarium. Seawater was aerated and maintained at conditions approximating those of a tropical coral reef (27.2 ± 0.4 °C, 34.6 ± 0.4 ppt, pH = 8.10 ± 0.04) and clams were exposed to a 2h dusk: 8h light: 4h dusk: 10h dark photocycle throughout acclimation. Mean irradiance during the light cycle was 88.82 ± 1.08 234.65 ± 2.45 µEinsteins m^-2 s^-1. Clams were held for 25 days prior to measurement of metabolic rates followed by tissue fixation. All clams were sampled during the light cycle for metabolic rate and VHA localization experiments.

For VHA inhibition experiments involving concanamycin A, adult T. maxima (n = 4; shell length 17.2 ± 6.0 cm) were collected from fringing reefs around the island of Mo’orea in the Society Archipelago, French Polynesia during October 2015. For bafilomycin A1 experiments, adult T. maxima clams (n = 3; shell length 15.0 ± 2.6 cm) were again collected from the same fringing reef sites during October 2016. In both studies, clams were held for 14 days prior to use in the experiment in an outdoor, flow-through seawater system, which drew water from the adjacent fringing reef.

Antibodies and western blot analysis

Custom rabbit anti-VHA polyclonal antibodies recognize a conserved epitope in the VHA_B subunit, AREEVPGRRGFPGY. These antibodies specifically recognize VHA_B by Western blot and immunofluorescence in diverse taxa including scleractinian corals (Barott et al. 2015), polychaete worms (Tresguerres et al. 2013), mussel (Thomsen, J., Himmerkus, N., Holland, N., Sartoris, F. J., Bleich, M. and Tresguerres 2016), hagfish (Clifford et al. 2015) and sharks (Roa et al. 2014).

Juvenile T. maxima (n=3) were vivisected and sections of gill, siphonal and byssal mantle tissues were frozen in liquid nitrogen and ground to a fine powder with a pestle in a ceramic mortar. 0.1 g of tissue was then combined with 500 µL of ice-cold S22 Buffer (450 mM NaCl, 10 mM KCl, 58 mM MgCl2, 10 mM CaCl2, 100 mM Hepes, pH 7.8) which contained a protease inhibitor cocktail (Sigma) and phosphatase inhibitor mix PhosStop (Roche Applied Science) and homogenized in a glass homogenizer. After a brief low-speed centrifugation to remove tissue debris, Symbiodinium cells were pelleted by centrifugation at 500 g for 10 min at 4 °C. The supernatant was then centrifuged again at 2100 g for 30 min (4 °C) and the “crude homogenate” supernatant fraction was removed from pelleted membranes. Symbiodinium and membrane-pellets were each resuspended in 100 µL of ice-cold homogenization buffer.
Protein concentration was determined in triplicate by Bradford Assay (Bio-Rad, Hercules, CA, USA). Western blotting was carried out following previously described methods (Roa et al. 2014). In brief, 5–25 μg of total protein were separated on polyacrylamide mini gel (60 V 15 min, 200 V 45 min) and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad). PVDF membranes were then incubated in blocking buffer (TBS-T with 5% non-fat powdered milk) at room temperature for 1 h before incubation in the primary antibody (30 μg ml⁻¹ in blocking buffer) at 4 °C overnight. PVDF membranes were washed three times in TBS-T and incubated in secondary antibody (1:10,000) at room temperature for 1 h. Bands were visualized through addition of ECL Prime Western Blotting Detection Reagent (GE Healthcare, Waukesha, WI, USA) and imaged and analyzed in a BioRad Universal III Hood using ImageQuant software (BioRad). PVDF membranes incubated in blocking buffer with anti-VHA antibodies and 10-fold excess blocking peptide served as control and did not show any bands.

**Immunolocalization of VHA**

For VHA to be playing an active role in regulating symbiont photosynthesis, we would predict it to be localized to membranes of the zooxanthellae tubules surrounding Symbiodinium cells. We utilized an immunohistochemical staining approach to test this predicted localization pattern in sections of T. maxima siphonal mantle tissue containing symbiotic algae. Sections of tissue roughly 1 cm x 1 cm x 0.5 cm thick were vivisected from juvenile clams (n=6) and placed on a shaker plate in a petri dish containing 0.1M sodium cacodylate buffer with 3% paraformaldehyde and 0.35% glutaraldehyde at 4 °C for 6 hours. Tissue was then transferred to 50% ethanol for 6 h, and finally stored in 70% ethanol until processed for immunohistochemistry. Tissues were then serially dehydrated in 95% ethanol (10 min), 100% ethanol (10 min), and xylene (3 x 10 min) before being transferred to paraffin (55 °C, 3 x 10 min) and left to solidify overnight. Paraffin-embedded tissues were sectioned at 12 µm using a rotary microtome and three consecutive sections were placed on glass slides placed on a slide warmer (37 °C) overnight. Paraffin was removed and sections were serially rehydrated in xylene (3 x 10 min), 100% ethanol (10 min), 70% ethanol (10 min), and permeabilized in 0.2% triton-x TBS-T in phosphate buffered saline (PBS) for 10 min. Tissue sections were blocked for 1 h in blocking buffer (0.2% triton-x, 2% normal goat serum, and 0.02% keyhole limpet hemocyanin in phosphate buffered saline, pH 7.8), and stained with the primary anti-VHA_B antibody (3 μg mL⁻¹ in blocking buffer) overnight at 4 °C. Sections were washed three times in PBS, incubated with the secondary antibody (1:500, goat anti-rabbit Alexa 555) for 1h at room temperature, and stained with Hoescht 33342 (Invitrogen, Grand Island, NY, USA) at 5 μg mL⁻¹ for 5 min to visualize host nuclei. Controls were prepared as above except sections were incubated with anti-VHA_B antibodies and 1000-fold excess (mol:mol) blocking peptide.

Immunofluorescence was detected with an epifluorescence microscope (Zeiss AxioObserver Z1 and Zeiss Apotome2) under metal halide illumination with appropriate filters. Digital images were adjusted for brightness and contrast only using Zeiss Axiovision software.

**Oxygen consumption and production rates**

All respirometric and photosynthetic measurements were conducted at 27 ± 1 °C using a stable temperature water bath. Oxygen consumption rates were measured fluorometrically, in the dark,
on freshly vivisected ~ 2 mm x 2 mm sections of siphonal mantle tissue immersed in seawater containing 1% DMSO in sealed glass vials (750 µL volume) using a PreSens OxyDish Reader (Loligo 1421-01). Controls were conducted with no tissue to correct for background oxygen consumption in the media. Concentration of dissolved oxygen was measured every 10 s for 5 min and oxygen consumption over time was estimated by a linear regression of dissolved oxygen concentrations, normalized by mass and corrected with no-tissue control blanks. For a subset of tissue samples, oxygen consumption rates were also measured post-light-exposure to examine phototrophic effects on holobiont metabolic rate. For these samples, tissues were prepared as above, but were incubated in bright light supplied by a 28W fluorescent light source (Coralife model 5300, 10K/420nm actinic bulbs) with tissue exposed to an average irradiance of 234.65 ± 2.45 µEinsteins m$^{-2}$ s$^{-1}$ as measured with a PAR scalar irradiance sensor (Biospherical Instruments, Model QSL2101; x̅ ± SD) for 20 min prior to measuring dissolved oxygen concentrations in the dark.

Inhibition of VHA activity was carried out using the specific inhibitors bafilomycin A1 and concanamycin A as has been performed previously (Barott et al. 2015). The effects of bafilomycin A1 on photosynthesis were assayed as described for metabolic rate above with slight modifications. Tissue samples were arranged for maximal surface area illumination and were incubated with 1% DMSO in open vials of seawater under constant illumination for 10 mins before recording began at 30 s sampling intervals. Media was then replaced with either 1% DMSO in seawater as a carrier control or a 1 µM solution of the VHA inhibitor bafilomycin A1 in 1% DMSO and tissues were allowed to incubate for 15 mins under the same initial conditions before recording began again at 30 s sampling intervals for 10 min.

To measure the effect of concanamycin A on gross oxygen production rates in the field under ambient tropical insolation, we used an end-point respirometric approach. Siphonal mantle tissues from freshly vivisected adult clams (n = 4) were placed in 1.5 mL microcentrifuge tubes and filled completely with either 1% DMSO in seawater or 1 µM concanamycin A1 in 1% DMSO in seawater. Half of the samples were wrapped with aluminum foil to allow for measurement of respiration rates, and no-tissue controls were used to correct for background oxygen production as described previously. All samples were transferred to outdoor holding tanks at 27 °C and experiments were performed under ambient solar irradiance (~14:00 – 15:30 h). Tissues in the photosynthetic treatments were continuously exposed to ~ 107,026.3 ± 21,604.1 Lux (~ 1,980 ± 400 µmol photons m$^{-2}$ s$^{-1}$; x̅ ± SD) as measured with a Mastech Digital Lux Meter (MS6612) for 25 mins prior to measurement of end-point concentration. After 25 mins of exposure, microcentrifuge tubes were gently rocked to mix and samples of media were quickly pipetted into the glass vials of the OxyDish reader which were immediately sealed. Concentration of dissolved oxygen was measured for 3 mins in each vial and averaged to give final end-point oxygen concentrations for each treatment. Gross oxygen production rates were calculated as the mass-normalized rate of oxygen production in the light-exposed treatments plus the mass-normalized oxygen consumption rates of the dark treatment for each tissue.
RESULTS AND DISCUSSION

VHA is highly abundant in T. maxima siphonal mantle tubules

Western blot analyses revealed very high VHA abundance in siphonal mantle, and much lower abundance in Symbiodinium and clam byssal mantle or gill (Fig. S1-3) where VHA may be involved in hemolymph acid-base homeostasis and ammonia secretion as suggested in other molluscs (Thomsen, J., Himmerkus, N., Holland, N., Sartoris, F. J., Bleich, M. and Tresguerres 2016). Immunofluorescence staining in siphonal mantle showed VHA is highly expressed in the apical membrane of epithelial cells of gut-derived tubules containing Symbiodinium (Fig. 2). The strong localization and high concentrations of VHA in tissues containing high Symbiodinium levels suggests an active role of this class of proteins in regulating symbiont photosynthesis.

Clam VHA promotes Symbiodinium photosynthesis

To study the potential role of VHA in promoting Symbiodinium photosynthesis, we tested the effect of the specific inhibitors bafilomycin A1 and concanamycin A (Dröse and Altendorf 1983) on net O₂ production by isolated siphonal mantle tissue. Both VHA inhibitors significantly reduced oxygen production compared to controls (Figs. 3 and 4), suggesting that VHA actively promotes photosynthetic production in hosted Symbiodinium algae. In siphonal mantle samples exposed to artificial illumination, bafilomycin A1 reduced gross oxygen production by 28% (Student’s t-Test, P < 0.05; Fig. 3B). Because photosynthetic efficiency is sensitive to light intensity (Falkowski and Raven 2007), we also tested the effects of inhibition of VHA on siphonal mantle samples exposed to full ambient tropical insolation in order to estimate photosynthetic productivity under conditions mimicking those on the reef. In these trials, concanamycin A also reduced net oxygen production rates, in this case by 37% (Student’s t-Test, P < 0.001; Fig. 4). These results demonstrate that, as in scleractinian corals, giant clam VHA activity regulates symbiont photosynthesis, greatly increasing photosynthetic productivity.

The likely mechanism behind this increased productivity is increased supply of inorganic carbon substrates for Symbiodinium photosynthesis, as has been suggested previously (Barott et al. 2015). In Symbiodinium, the fixation of inorganic carbon into organic forms is conducted primarily by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) which has a low affinity for CO₂ as a substrate (Rowan et al. 1996). Thus, CO₂ must be present at high concentration to permit Symbiodinium photosynthesis (Rowan et al. 1996). This feature of Symbiodinium necessitates the presence of a carbon-concentrating mechanism (CCM) within host tissues in order to ensure adequate supply of carbon to the algae. We postulate that the primary source of carbon fueling Symbiodinium photosynthesis is respiratory CO₂ from the host clam which, through the action of carbonic anhydrase, also serves as the likely source of H⁺ for VHA activity (Fig. 1B).

Under this model of carbon supply, free H⁺ from metabolically formed and dissociated carbonic acid (HCO₃⁻ + H⁺) could be actively transported across siphonal mantle cellular membranes by VHA into the lumen of the zooxanthellae tubules (Fig. 1B). This acidification would in turn generate an electromotive force facilitating concomitant transport of HCO₃⁻ into the
zooxanthellae-filled tubules. Although the presence of these bicarbonate transporters is inferred from the increased rate of carbon fixation within Symbiodinium in the presence of VHA, their identity remains to be determined. Under the acidic conditions within the tubule lumen, host-derived carbonic anhydrase could facilitate the conversion of HCO$_3^-$ to CO$_2$, which, through diffusion, would increase the partial pressure of CO$_2$ within zooxanthellae chloroplasts, thus permitting efficient photosynthesis despite the low-affinity of Symbiodinium RuBisCO (Tresguerres 2016). The resulting O$_2$ freely diffuses back into the host clam tissue, where it fuels an increased aerobic metabolic rate (as discussed below) further supplemented by reduced carbons translocated from Symbiodinium. As in coral however, the mechanisms underlying translocation of sugars from algae to host remain to be explored.

**Upregulation of aerobic respiration rate in the light**

Previous work has shown that the vast majority of sugars fixed by Symbiodinium are transferred to the giant clam host where they are used to sustain metabolism and growth (Klumpp and Griffith 1994; Klumpp and Lucas 1994). Here, we report that Symbiodinium photosynthetic activity also enhances host aerobic respiration. Mass-normalized oxygen consumption rates of siphonal mantle tissue biopsies measured at 27 °C in the dark were $0.022 \pm 0.009 \, \mu$mol O$_2$ g$^{-1}$ min$^{-1}$ ($\bar{x} \pm$ SEM) in juvenile T. maxima (Fig. 5). This rate was indistinguishable from the rate of $0.022 \pm 0.002 \, \mu$mol O$_2$ g$^{-1}$ min$^{-1}$ measured in the same tissue at the same temperature in samples from field-acclimatized adults (Student’s t-Test, P > 0.05). However, oxygen consumption rates dramatically increased to $0.107 \pm 0.021 \, \mu$mol O$_2$ g$^{-1}$ min$^{-1}$ ($\bar{x} \pm$ SEM) in juveniles after 20 minutes of exposure to bright light (Fig. 5). These results demonstrate that light-driven symbiont productivity fuels an increased aerobic metabolic rate in host clams and enhances holobiont oxygen-consumption by nearly 490% (Fig. 5). To what degree this increased metabolic rate stems from an increased supply of carbonate substrates for catabolism or an increased partial pressure of O$_2$ within the haemolymph and tissues remains to be explored. However, the rapid rise in metabolic activity (occurring within just 20 min of exposure to light) suggests increased availability of O$_2$ within host tissues as the likely driver of increased metabolic rate rather than translocated sugars.

**Broader significance**

We demonstrate that VHA-driven CCM enhances Symbiodinium photosynthetic productivity in giant clams, and thus plays an important role in regulating holobiont energy availability. In addition, VHA-driven transport of carbon from the host clam to algal symbionts plays a vital role in maintaining acid-base balance within the host clam. Previous research has shown that in symbiotically intact giant clams, zooxanthellae are capable of completely removing inorganic carbon from the clam haemolymph and significantly increasing haemolymph pH (Rees et al. 1993). However, in bleached clams, concentration of inorganic carbon in the haemolymph can rise above that of the surrounding seawater, resulting in significant tissue acidosis (Leggat et al. 2003). This suggests that not only does clam respiratory CO$_2$ fuel Symbiodinium production, but that without algal photosynthesis, giant clams are incapable of efficiently disposing of excess inorganic carbon from their tissues (Leggat et al. 2003). VHA-driven CCM in the zooxanthellae tubules likely facilitates this rapid drawdown of haemolymph pCO$_2$ in tridacnid clams and could
have important implications for how giant clams respond to increasing temperature and oceanic pCO$_2$ resulting from anthropogenic climate forcing.

By the end of the century, global temperature and oceanic pCO$_2$ are predicted to rise by 4 ºC (global warming) and > 500 ppm (ocean acidification) respectively, with adverse consequences for many marine species (Watson et al. 2012; Watson 2015). Confronted with both direct exposure to rising aqueous pCO$_2$ and temperature-induced increases in metabolic CO$_2$ production, marine organisms will likely face increasing inorganic carbon loads within their tissues potentially compromising maintenance of acid-base balance (Gazeau et al. 2013). However, VHA-driven CCM in giant clams permits the shuttling of inorganic carbon from within host tissues to Symbiodinium, and thus may significantly ameliorate the effects of environmentally-driven tissue acidosis. A recent investigation of the effects of elevated pCO$_2$ on giant clams provides evidence for this hypothesis. Watson (2015) demonstrated that, under conditions of low ambient light, exposure to increased pCO$_2$ congruent with predictions for the year 2100 resulted in reduced growth rates and significant mortality among juvenile tridacnid clams (Watson 2015). However, these negative effects were strongly counteracted (i.e. no observed mortality and reduced effects on growth) when clams were exposed to high light conditions demonstrating that Symbiodinium photosynthesis significantly altered host response to acidification (Watson 2015). This strongly implies a positive role of algal photosynthesis in removing excess carbon from host tissues with VHA CCM as the likely mechanism of carbon transfer. Thus, VHA-driven CCM within giant clams likely acts as a physiological buffer against acidification- and temperature-induced stress and may significantly ameliorate the negative effects of these climate drivers on these species.

In addition to the important roles VHA may play in mitigating the negative effects of climate change in tridacnid clams, we propose that the similar pattern of expression, localization, and functionality of VHA within corals and giant clams, despite differing anatomies, serves as an example of convergent exaptation across taxa which live symbiotically with Symbiodinium algae. Both groups of organisms have utilized VHA to overcome physiological constraints to photosynthesis, including the need for a CCM. In addition, the differential localization of VHA at sites of calcification and photosynthesis in both corals and giant clams suggests that this one class of protein has been repurposed for multiple, similar, physiological functions in both taxa. Given the significant energetic benefits VHA activity has for the host coral and clam, we interpret these findings as a clear example of convergent exaptation in marine photosymbioses. Further research is necessary to discern whether VHA ultimately acts to transport H$^+$ from sites of calcification to symbiotic algae in both corals and tridacnid clams, but our results conclusively demonstrate that both taxa utilize VHA to enhance carbon fixation.

Despite meticulous early research efforts which quantified the surpassingly large contribution of dinoflagellate symbionts to their hosts daily metabolic needs (Klumpp and Griffith 1994), the question still remains, what is the ultimate fate of this “excess” fixed carbon? In scleractinian corals, as much as half of the carbon gained from zooxanthellate symbionts is estimated to be lost to the environment in the form of mucus which is excreted nearly continuously by the cnidarian host (Crossland et al. 1980; Edmunds and Davies 1986; Leletkin 2000). Whereas giant clams also produce copious amounts of mucus, the majority is utilized in particulate matter capture at the gills and in transport of ingested material in the digestive tract and thus is likely
quickly resorbed by the host clam (Urrutia et al. 2001). The major loss of mucus to the environment in bivalves occurs in the production of pseudofeces (Urrutia et al. 2001). However, the volume of mucus lost from clams in pseudofeces is significantly less than that loss in the mucus sheets of corals and likely represents a negligible component of the global energy budget in bivalves (Urrutia et al. 2001). With no other obvious sink for excess fixed carbon, we speculate that the majority of surplus photosynthates are directed towards massive growth (i.e. the phenomenon of gigantism) within giant clams.

Gigantism has frequently arisen from symbiotic associations between animals and chemo- or phototrophic microbes or algae (Childress and Girguis 2011; Vermeij 2013, 2016). The prevailing hypothesis is that adoption of a symbiotic partnership benefits hosts through an increased energy supply for growth, augmenting food availability through symbiont chemo/photosynthetic production (Vermeij 2016). We provide supporting evidence for that hypothesis here and show that in T. maxima symbiont photosynthetic production greatly alters host metabolic rate, substantially increasing energy turnover (Fig. 5). Whereas the first chemosymbiotic bivalves were small (mytilids of the Late Eocene, < 50 mm shell length), we hypothesize that adoption of a photosymbiotic lifestyle in the tridacnid lineage ultimately served as an enabling feature permitting the evolution of gigantism in this group (Vermeij 2016).

CONCLUSION

In this study, we demonstrate that giant clams of the genus Tridacna regulate and promote symbiont photosynthesis through the activity of vacuolar-type H^+ -ATPase. We confirm that giant clam VHA is expressed predominantly in siphonal mantle in T. maxima where it is localized in close proximity to symbiotic zooxanthellae. We demonstrate that VHA actively promotes algal photosynthesis, increasing rates of O_2 production by 37% and that photosynthetic activity increases holobiont metabolic rate by as much as 490%. Taken together, these results suggest that localization and activity of VHA within siphonal mantle of giant clams constitutes an exaptation in the tridacnid lineage, convergently shared by reef-building corals, which confers strong, positive energetic benefits to the host and may have permitted the evolution of gigantism within this group of bivalves.

ACKNOWLEDGEMENTS

We are grateful to Yuzo Yanagitsuru for help with Western blots and to Camilla Souto for help in collecting specimens of T. maxima in the field. E.A. designed this study, collected specimens and generated most data. E.A. and M.T. composed this paper. E.A., J.R. and M.T. carried out the analysis of VHA. J.S. and M.T. advised on experimental design and interpretation. All authors critically read and edited the manuscript.
REFERENCES


Norton JH, Jones GW (1992) The giant clam: an anatomical and histological atlas. Australian Centre for International Agricultural Research, Canberra, Australia, Canberra


FIGURE CAPTIONS

Figure 1. Histological diagrams of Tridacna maxima siphonal mantle tissue and symbiont arrangement. (A) T. maxima siphonal mantle ultrastructure showing approximate arrangement of mantle margin cells (M) relative to light-refractive iridocytes (I), zooxanthellae tubules (ZT) and Symbiodinium (S). Anti-VHA\textsubscript{B} staining in apical margins of siphonal mantle cells is shown in green, and dotted line (*) denotes cross section of zooxanthellae tubule displayed in (B). (B) Model of zooxanthellae tubule system in T. maxima as compared to endosymbiotic arrangement in corals. In giant clams, Symbiodinium are hosted extracellularly in digestive tubules (ZT) bounded by host clam cells (blue) that express VHA in apical membranes (green). In corals, algae are housed intracellularly in a membrane-bound symbiosome (SB) containing VHA. In both taxa, metabolic CO\textsubscript{2} is converted by host carbonic anhydrase (CA) to H\textsuperscript{+} and HCO\textsubscript{3}\textsuperscript{-} (1). H\textsuperscript{+} is then actively transported into the tubule lumen by clam VHA (green/yellow icon; 2). HCO\textsubscript{3}\textsuperscript{-} follows via an unknown bicarbonate transport mechanism (grey circle; 3), whereupon putative host-derived CA catalyzes the reconversion of both substrates into CO\textsubscript{2} (4). CO\textsubscript{2} diffuses into Symbiodinium cells (5) where it is transported by unknown mechanisms to algal chloroplasts. Algal photosynthesis drives the production of O\textsubscript{2}, which diffuses into host tissues, and sugars (6) which are translocated by unknown mechanisms to the host clam.

Figure 2. Immunolocalization of vacuolar-type H\textsuperscript{+}-ATPase in tissues of the giant clam T. maxima. Negative control omission of primary antibody (A) in siphonal mantle, and corresponding anti-VHA\textsubscript{B} antibody staining in byssal (B) and siphonal mantle (C). High magnification image of the zooxanthellae tubule system shown in (D). VHA in green, nuclear DAPI staining in blue, and chlorophyll autofluorescence in red. ZT, zooxanthellae tubules; M, shell-mantle margin.

Figure 3. Effects of VHA-inhibition by bafilomycin A1 on net photosynthetic oxygen production in T. maxima. Representative oxygen production traces from T. maxima before and after addition of (A) DMSO and (B) 1 µM bafilomycin A1. Breaks in data correspond to pauses in data acquisition for treatment addition and dotted lines indicate initial photosynthetic production trajectories. (B) Relative net oxygen production rates in the presence of DMSO or concanamycin A. Data are plotted as mean rates with SEM and asterisk denotes significant difference from control.

Figure 4. Effects of VHA-inhibition by concanamycin A on net photosynthetic oxygen production in T. maxima. Displayed data are net oxygen production rates in the presence of DMSO or concanamycin A relative to average DMSO control. Data are plotted as mean rates with SEM and asterisk denotes significant difference from control.

Figure 5. Light-induced alteration of metabolic performance in T. maxima. Rates of oxygen consumption in the dark in tissue incubated in DMSO either in the absence of light (dark) or after 20 minutes of exposure to light. Data are plotted as mean rates with SEM and asterisk denotes significant difference between treatments.
FIGURES

Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
APPENDICES

Appendix A.1 – Supplemental Figure Captions

Figure S1. Western blots for total protein in (A) crude homogenates of T. maxima tissue and (C) Symbiodinium and siphonal mantle membrane fractions (with 5-25 µg protein loaded) using anti-VHA\textsubscript{B} antibodies and (C) control using anti-VHA\textsubscript{B} antibodies incubated overnight with 10-fold antigen peptide. SM, siphonal mantle; BM, byssal mantle; G, gill; Z, zooxanthellae; M, siphonal mantle membrane.

Figure S2. Western blots for total protein in (A) Symbiodinium and (B) siphonal mantle membrane fractions using anti-VHA\textsubscript{B} antibodies and (C) control using anti-VHA\textsubscript{B} antibodies incubated overnight with 10-fold antigen peptide. Z, zooxanthellae; M, siphonal mantle membrane.

Figure S3. Immunofluorescent staining for vacuolar-type H\textsuperscript{+}-ATPase in T. maxima gill tissue. VHA in green, nuclear DAPI staining in blue, and chlorophyll autofluorescence in red. ZT, zooxanthellae tubules; M, shell-mantle margin
Figure S1.

A

<table>
<thead>
<tr>
<th>SM</th>
<th>BM</th>
<th>G</th>
</tr>
</thead>
</table>

B

<table>
<thead>
<tr>
<th>SM</th>
<th>BM</th>
<th>G</th>
</tr>
</thead>
</table>

250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
Figure S2.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z</td>
<td>Z</td>
<td>Z</td>
</tr>
<tr>
<td></td>
<td>15 µg</td>
<td>15 µg</td>
<td>25 µg</td>
</tr>
<tr>
<td></td>
<td>5 µg</td>
<td>15 µg</td>
<td>5 µg</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>M</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 2  Elevated temperature, but not acidification, reduces fertilization success in the small giant clam, *Tridacna maxima*.

Eric J. Armstrong

ABSTRACT

Elevated temperature and decreased oceanic pH (ocean acidification) associated with anthropogenic climate change are known to adversely affect fertilization success and development in marine invertebrates. However, estimates of these impacts remain unresolved for many ecologically and economically important species including giant clams of the genus *Tridacna*. I investigated the individual and interactive effects of warming and acidification on fertilization (successful first cellular cleavage) in the economically important bivalve mollusc, *Tridacna maxima* in Mo’orea, French Polynesia. Fertilization was conducted under ambient conditions (27 °C, pH 8.1) and under temperature and pH conditions projected for the coming century (31 °C, pH 7.6). Fertilization success was low under ambient conditions (48.4 ± 3.0%) and was significantly reduced under elevated temperature, alone and in combination with high pCO$_2$ (18.8 ± 4.3 % and 16.4 ± 4.0% respectively). However, acidification alone had no effect on fertilization success in *T. maxima* (47.5 ± 2.9%). Fertilization in *T. maxima* was resilient to reduced pH, but was strongly inhibited by elevated temperature. Populations of *T. maxima* may therefore be at risk of low reproductive success over the coming century as a result of rising oceanic temperature.

INTRODUCTION

Elevated oceanic temperatures (ocean warming) and reduced oceanic pH (ocean acidification) are products of increasing atmospheric pCO$_2$ and have been shown in many taxa to alter developmental timelines (Stumpp et al. 2011), inhibit growth (Stumpp et al. 2011; Dorey et al. 2013), deform calcitic exoskeletons (Gazeau et al. 2013), and reduce larval fitness (Kroeker et al. 2010). However, the magnitude of these effects can vary significantly both across taxa (Kroeker et al. 2010; Harvey et al. 2013) and across life history stages within a species (Kurihara 2008; Harvey et al. 2013).

Early life history stages are often more susceptible to acute changes in the environment than juveniles or adults and require a narrower range of conditions to complete their development (Kurihara 2008; Byrne and Przeslawski 2013). Mortality in such stages of broadcast spawning organisms can be extremely high (> 90%) even under ambient natural conditions (Kurihara 2008) and thus sensitive early life history stages may represent important bottlenecks for
population growth and persistence under a changing climate (Byrne & Przeslawski 2013). Recent studies have repeatedly demonstrated heightened sensitivity of early developmental stages of marine organisms to several environmental drivers including increased temperature and reduced pH (Kurihara 2008). However, for many species, we lack data regarding physiological sensitivity of early life stages to these potential stressors. Among these understudied taxa are the so-called giant clams of the genus Tridacna, which are among the most charismatic inhabitants of tropical coral reefs and are important both culturally and economically throughout their range (Neo et al. 2015).

Giant clams are highly fecund, seasonal broadcast spawners that inhabit tropical Indo-Pacific reefs (Knop 1996) and are harvested both locally and commercially for the seafood and ornamental markets (Firdausy and Tisdell 1992). Giant clams are protandric hermaphrodites that release their gametes sequentially, first spawning sperm first with egg release occurring several minutes afterwards (Wada 1952, 1954). This reproductive behavior is uncommon among hermaphroditic bivalves, including most other cockles, which generally discharge both sperm and eggs simultaneously (Soo and Todd 2014). In addition, populations of giant clams exhibit synchronized spawning across wide stretches of reef habitat, but reproductive cues in giant clams remain unresolved (Soo and Todd 2014). Seasonal and episodic periods of decreased temperature (often associated with increased flow across lagoonal reefs) have been implicated as initiators of spawning in some populations (Van Wynsberge et al. 2016). Gamete release is also exceptional in giant clams and several million gametes are released in one reproductive event earning giant clams the moniker of “pinnacle of fecundity in the animal kingdom” (Yamaguchi 1977; Lucas 1994). Despite this prolific reproductive output, however, the fertilization window in giant clams is relatively limited and gametes are only viable for 4–6 h after release (Tan and Yasin 2001). Furthermore, coral reef habitats, the settlement site for larval giant clams, are currently experiencing swift changes in abiotic conditions including unprecedentedly rapid rises in temperature (Hoegh-Guldberg 1999), with severe negative consequences for foundational species including corals themselves (Hoegh-Guldberg, 1999; Anthony et al., 2008; Marshall & Baird, 2009; De’ath et al., 2012) and, potentially, giant clams (Watson et al., 2012).

While much has been learned about early development of giants clams in mariculture (Beckvar 1981; Crawford et al. 1986; Lucas 1994; Toonen et al. 2011), no studies have directly examined the sensitivity of giant clam syngamy success, or eff fertilization, to predicted warming and acidification associated with global climate change. Because giant clams are relatively scarce, long-lived, and slow to mature, they may exhibit limited capacity for adaptation to the rapid environmental changes associated with anthropogenic forcing and may thus be particularly vulnerable to the effects of ocean warming and acidification. With global temperature expected to rise by +4 °C and average pH of the Indo-Pacific reefs expected to decrease 0.5 units (to ca. 7.7) by the end of the century (IPCC, 2014; IOC, SCOR, and IGBP 2013), there is a strong need to examine the physiological effects of these changes on early development in giant clams. Here, I present the first data documenting responses to these drivers in early life history stages of a giant clam, the economically and ecologically important “small” giant clam, Tridacna maxima (Röding, 1798).
METHODS

Broodstock Acquisition and Maintenance

Eight adult broodstock Tridacna maxima clams, shell length 12.2 ± 2 cm, were collected from areas of fringing reef around the islands of Mo’orea and Tahiti, French Polynesia during September and October 2016. Broodstock clams were immediately transferred to an onshore holding tank (~ 380 L) at the University of California, Berkeley, Richard B. Gump Field Station where they were maintained under flow-through seawater conditions (26.9 ± 1°C, 35.96 ± 0.2 PSU, pH = 8.44 ± 0.01) and ambient insolation for at least 2 days prior to use in the spawning experiment.

Spawning Induction

Because not all individuals released both eggs and sperm, spawning was carried out in four rounds in which gametes from two broodstock clams were crossed in one direction (i.e. one nominal “mother” per round). In each spawning there were two clams; one clam was used for eggs and the other clam was used for sperm. Four spawnings were conducted at intervals of approximately 3 days yielding a total of n=4 biological replicates. Spawning was induced in each clam by injection of serotonin (5-hydroxytryptamine creatine sulfate complex (Sigma-Aldrich CAS 153-98-0; prepared as 0.5 mL of 1 mM serotonin in 2 µm-filtered seawater) directly into the gonad (Crawford et al. 1986). Clams were placed in separate 1.5 L spawning tanks where they released sperm. Concentrated sperm stock was collected from one clam (i.e. nominal “father”) using a 50 mL syringe positioned above the exhalant siphon. After sperm production had ceased, clams were then transferred to new 1.5 L egg collection tanks and allowed to continue gamete release until spent and eggs were collected from another individual (i.e. nominal “mother”).

Fertilization and Zygote Culture

For each nominal mother (n = 4), one 50 mL sample of egg stock was aliquoted prior to fertilization and stored for quantification of egg density. Eggs were counted and imaged from ten, 0.1 mL aliquots of each stock solution using a Leica® dissecting microscope with optical light microscope camera (Leica MZ16; Leica DFC420). Images were also taken of eggs alongside scale references, and egg diameter was measured using the image analysis program ImageJ64 (Schneider et al. 2012).

Culture water for fertilization was collected from offshore, and filtered through a 75 µm mesh before being delivered to four 80 L header tanks at a rate of ca. 50 L hr⁻¹. Parameters in header tanks were maintained following a 2x2 temperature (28 ± 1°C modern ambient, 31 ± 2°C predicted future) by pH (7.6 ± 0.06 and 8.1 ± 0.04) design. Elevated temperature was achieved using 250 W aquarium heaters and high pCO₂ conditions were maintained as described in the
Seawater Acidification section below. A full list of experimental seawater parameters is given in Table 1.

Fertilization took place under treatment conditions. Eggs were washed twice with treatment water before twelve, 50 mL aliquots of egg stock solution were transferred to individual 50 mL Falcon tubes modified with cap-mounted driplines and 75-µm mesh covered bottoms, permitting flow-through culturing, hereafter referred to as culture tubes. For each paternal/maternal pair, three replicate culture tubes (technical replicates) were used for each of the four temperature x pH treatments. Culture tubes were placed in separate 1.7 L water baths containing respective treatment water. Fertilization was initiated by the addition of 1 mL of well-mixed, concentrated sperm stock from the paternal clam and tubes were sealed allowing fertilization to take place under treatment conditions. Culture water was delivered from header tanks via cap-mounted driplines (7.6 L hr⁻¹ flow rate) to the culture tubes. Tubes were continuously immersed and kept in a semi-shaded location outdoors. Embryos (which were larger than tube mesh coverings and therefore retained) were maintained in the flow-through culture tubes for 2-hours prior to estimation of fertilization success.

Assessment of Fertilization Success

Fertilization success was measured as the number of zygotes that had successfully undergone cleavage to the 2-cell stage 2 h post-fertilization. To count successfully dividing embryos, inflow from header tanks was stopped and culture tubes were gently agitated in their respective water baths to re-suspend eggs. Three, 1 mL aliquots were taken from each culture tube and transferred to separate microcentrifuge tubes from which three subsequent 100 µL subsamples were counted. This resulted in a total of nine replicate counts for each culture tube and 36 counts per temperature x pH treatment x spawning pair. Aliquots were placed on a glass microscope slide for visual inspection of embryos. All embryos within a 100 µL aliquot were counted and the proportion of embryos which had successfully undergone first cellular cleavage was recorded.

Statistical Analyses

Data were analyzed using the statistical software program R (v 3.2.5; R Development Core Team, 2008). Normality of data was checked using the shapiro.test and test_normality functions of the “stats” and “LambertW” packages. Fertilization success data were not normally distributed as a result of the low fertilization success recorded in pairing 2. While removal of pairing 2 data did cause remaining data to meet normality criteria, it did not alter the results of statistical analyses (i.e. significant factors remained unchanged). Thus, all response variables were analyzed using a linear mixed effects model, including all interaction terms, with pH, temperature, and pairing (maternal/paternal pair) as fixed effects and culture tube as a random effect (DF =98). Results of this analysis are given in Table 2.

Seawater Acidification and Carbonate Chemistry
Elevated pCO$_2$ treatments were maintained by controlled bubbling of 80 L header tanks with pure CO$_2$ using an IKS Aquastar pH controller and solenoid-valve gas regulation system (CO$_2$ Art). Seawater pH$_T$ and salinity were measured every 15 minutes using a Professional Plus Multiparameter Instrument (YSI Quatro Dual, Model 1001 pH sensor) calibrated with four, spectrophotometrically determined, pH samples. Calibration standards were measured using m-Cresol Purple sodium salt dye using an Evolution 60S UV-Visible spectrophotometer against a Tris-buffered pH reference standard (Dickson Strand 13/Bottle 74) following modified best practice methods (Dickson et al. 2007). Seawater temperature was recorded using Thermochron iButton data loggers (± 0.5 °C resolution; model DS192G-F5#; iButton Link Technology). Samples for determination of total alkalinity (A$_T$) were taken immediately prior to fertilization and were measured via open-cell potentiometric titration with an automatic titrator (T50, Mettler-Toledo). Measurements of A$_T$ were conducted on duplicate 50 mL samples at room temperature (~23 °C) and A$_T$ was calculated as described previously (Dickson et al. 2007). Titrations of certified reference materials (CRM) provided by A. G. Dickson (Dickson Batch 13/Bottle 74) yielded A$_T$ values within ± 4 mol kg$^{-1}$ of the nominal value (n = 8). Parameters of the seawater carbonate system were calculated from salinity, temperature, A$_T$ and pH$_T$ using the R package seacarb (Table 1; Gattuso et al. 2015).

RESULTS

Reproductive Output and Morphology

Average reproductive output was estimated at 435,000 ± 58,000 (x̅ ± se) eggs ind.$^{-1}$. Egg cells were 47.4 ± 2.1 µm in diameter (x̅ ± sd) while jelly coats were ca. 16.4 ± 2.1 µm thick giving eggs a total diameter of ca. 84.0 ± 1.2 µm. The vast majority of eggs were well-formed and possessed complete jelly coats (Fig. 1).

Fertilization Success

Under ambient conditions, fertilization success in T. maxima embryos was 48.4 ± 3.0 % (x̅ ± 95%-ci; Fig. 2). Acidification did not affect fertilization success, as 47.5 ± 2.9% of eggs were fertilized under reduced pH conditions (Table 2). However, exposure to elevated temperature (either alone or in combination with reduced pH) resulted in a 30% decrease in fertilization success relative to the ambient conditions (18.8 ± 4.3 % and 16.4 ± 4.0 % respectively; Fig. 2; Table 2). There was no significant interactive effect between elevated temperature and high pCO$_2$ or between high pCO$_2$ and pairing (individual male/female pairs) on fertilization success in T. maxima (Table 2). However, there was a significant interactive effect between temperature and pairing (Table 2) with one female exhibiting an extreme negative response to elevated temperature (i.e. 0% fertilization success, Fig. 3).
DISCUSSION

In this study, I assessed the effects of elevated temperature and high pCO$_2$ concentrations on early development in the giant clam Tridacna maxima. My data are the first to show that fertilization success in a species of giant clam is negatively impacted by climate change drivers. Exposure to elevated temperature, alone or in combination with elevated pCO$_2$ resulted in a ca. 66% decrease in successful fertilization (i.e. fertilization rate of 17%; Fig. 2). This sensitivity of reproductive success to elevated temperature is likely to result significant negative consequences for persistence of giant clam populations under future ocean warming.

Sensitivity to Warming

The demonstrated sensitivity of fertilization success in giant clams to increased temperature stands in stark contrast to responses reported for other broadcast spawning organisms (e.g. corals, polychaetes, echinoderms) including several other species of bivalve molluscs (Crassostrea gigas, Mytilus galloprovincialis, Saccostra glomerata, and Spisula solidissima) (Byrne 2011b). In these species, high rates of fertilization were achieved over a broad range of ambient temperatures including warming scenarios far in excess of predicted future ocean conditions (up to + 6°C; M Byrne, 2011). In general, increased temperature reduces seawater viscosity, increases sperm metabolism and swimming speed, and facilitates the acrosome reaction, all of which have been shown to aid fertilization success in marine organisms (Kupriyanova, E.K. Havenhand 2005). In giant clams however, even the +3 ºC warming applied in this study was enough to significantly reduce fertilization success (Fig. 2). This is surprising given the routine use of heat shock (often conducted at temperatures of + 6 ºC above ambient) to obtain fertile gametes in giant clams (ACIAR 1992). However, after gametes have been spawned and collected, fertilization of giant clam eggs in aquaculture settings is universally conducted at ambient temperature, which may explain the success of this technique in generating healthy larvae despite exposure of adult clams to temperatures far in excess of those applied in this study. Furthermore, in the wild natural spawning in several populations has been linked to periods of decreased ambient temperatures (Van Wynsberge et al. 2016) suggesting that populations may have evolved temperature-responsive gamete release strategies to avoid exposing gametes to periods of increased temperature and thus maximize fertilization success. However, decreases in ambient temperature are by no means a universal spawning cue among giant clams (LaBarbera 1975; Jameson 1976; Beckvar 1981) and, despite considerable research, no consensus has yet been reached regarding initiation of reproduction in Tridacnid species (Soo and Todd 2014). Further research is certainly warranted to address the extreme temperature-sensitivity of giant clam fertilization, especially in light of both the natural history of reproductive timing in these species and the relative resilience of this response variable among many other bivalve molluscs (Byrne 2011b).
Resilience to Acidification

In contrast to giant clam’s demonstrated temperature-sensitivity, the relative insensitivity of giant clam fertilization to acidification is equally surprising. Reductions in ambient pH have been previously shown to negatively impact fertilization success in marine invertebrates, most notably in echinoderms where the process has been extensively studied (Kurihara and Shirayama 2004; Byrne et al. 2009). The mechanisms underlying this reduced fertilization success vary, but many result from direct negative effects of increased pCO$_2$ on sperm performance. For example, hypercapnia has been shown to reduce sperm swimming speed and thus impair fertilization (Havenhand et al. 2008; Morita et al. 2010), but the strength of this impairment differs across species. For example, in the sea urchin, Strongylocentrotus purpuratus, Christen et al. (1983) observed that low pH impaired sperm motility, but this was not similarly observed in either of two other species, Hemicentrotus pulcherrems and Echinometra mathaei (Kurihara and Shirayama 2004). In addition, Gregg & Metz (1976) indicated that low pH (6.5) inhibited the acrosomal reaction of sea urchin sperm in Arbacia punctulata, whereas in Anthocidaris crassipina, polyspermy was induced when eggs were fertilized at pH 7.0 and lower (Kobayashi 1973). While the mechanisms by which hypercapnia affects fertilization may vary, lowered pH certainly has demonstrated potential to reduce fertilization success in marine species.

However, despite these potential negative effects of hypercapnia on sperm swimming performance and egg-recognition, a relatively large number of acidification studies (17 across four phyla including several bivalve species and giant clams from this study) have demonstrated that fertilization is robust to pH as low as 7.4–7.6, values significantly lower than those projected for much of the ocean by the end of the century (Byrne 2011b). Byrne et al. have suggested that this resilience may reflect adaptation to environments in which pH is naturally highly variable (e.g. the intertidal or shallow subtidal zones; M Byrne, 2011). For example, comparison between two congeneric sea urchins demonstrated that, in the intertidal species, Heliocidaris erythrogramma, successful fertilization occurred under pH as low as 7.6 whereas in the subtidal congener, Heliocidaris tuberculata, fertilization was significantly impeded under similar pH conditions (Byrne et al. 2010). While the relative resilience of giant clam fertilization to reduced pH could potentially be explained, at least in part, by the relatively high, and consistent, daily variation in pH experienced on tropical coral reefs (as high as ± 0.3 units; Hofmann et al., 2011), not all reef-dwelling species are similarly robust. For example, in the tropical coral Acropora palmata, fertilization success under control pH conditions was similar to that observed in T. maxima (50% in A. palmata as compared to 48% in T. maxima), but fertilization was reduced by ca. 60% (i.e., 30% fertilization success) at pH 7.7 in the coral species (Albright et al. 2010). This suggests that exposure to natural pH variability alone may not explain the relative insensitivity of giant clam fertilization to acidification. More data from a greater diversity of species are needed to adequately address hypotheses regarding potential environmentally-driven low-pH adaptation of fertilization and to elucidate the mechanisms underlying this resilience.
CONCLUSION

While the robust nature of giant clam fertilization to predicted future ocean pH is encouraging, the strong susceptibility of this process to warming leaves open the possibility of severe negative population responses to ocean warming. Even under ambient conditions, survival in Tridacnid early life history stages is generally low, with reports of > 99% mortality over the course of development from zygote to juvenile metamorphosis (Beckvar 1981; Crawford et al. 1986). While not uncommon for highly fecund broadcast spawners, this low viability further highlights the potential of early-life history stages as “weak links” in giant clam population persistence. With populations of giant clams already severely overharvested over much of their range (Van Wynsberge et al. 2016), the additional impact of low fertilization success is likely to exacerbate population declines.

Restoration efforts are currently underway to utilize mariculture to restock giant clam populations on overexploited reefs (Neo and Todd 2013; Neo et al. 2013), but these efforts may ultimately be hampered by warming-induced infertility. In a recent study of the potential role of mariculture in reestablishing giant clam populations on the heavily exploited reefs of Singapore, captive-raised giant clams were successfully reestablished on highly impacted reefs sites, but no juveniles or recruits were ever found subsequently at restoration sites despite thousands of hours of surveying (Guest et al. 2008; Neo et al. 2013). This implies that successful larval recruitment from these captive-raised clams was virtually non-existent on Singapore’s reefs and represents a severe hurdle to the use of restocking alone as a means of reestablishing self-sustaining populations of giant clams on overexploited reefs (Guest et al. 2008). Larval recruitment failure could be a persistent problem if temperature-sensitive genotypes (as displayed in pairing 2 in this study; Fig. 3) are relatively common among wild populations of T. maxima. Given the extreme negative effects of even moderate warming on fertilization success in giant clams as demonstrated here, mariculture restoration efforts may ultimately be constrained by temperature sensitivity of syngamy in Tridacnid species.

ACKNOWLEDGEMENTS

I would like to thank Vaimiti Dubousquet for her tireless efforts in helping to both secure and spawn the clams utilized in this study. Thanks too to Antoine Puisay and Benoît Lemarechal, for help in collecting mature broodstock clams, and to Franck Lerouvreur, Pascale Ung, and Valentine Brotherson for their invaluable aid in construction and maintenance of the aquarium facilities and in securing CO₂ for use in this study. This research was conducted with US Government support to EJ Armstrong under and awarded by the Department of Defense, Air Force Office of Scientific Research, National Defense Science and Engineering Graduate (NDSEG) Fellowship, 32 CFR 168a.
REFERENCES


Georg M (2014) The Lambert Way to Gaussianize heavy-tailed data with the inverse of Tukey’s h-transformation as a special case.


Hofmann GE, Smith JE, Johnson KS, Send U, Levin L a, Micheli F, Paytan A, Price NN,


### Table 1. Measured and calculated seawater carbonate chemistry parameters. All parameters were calculated from salinity, temperature, total alkalinity ($A_T$) and pH (total scale) using the carb() function of the R package seacarb (Gattuso et al. 2015).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ambient pCO$_2$ Mean ± SD</th>
<th>High pCO$_2$ Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.02 ± 0.04</td>
<td>7.63 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>8.00 ± 0.10 (High T)</td>
<td>7.61 ± 0.11 (High T)</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>27.08 ± 0.8</td>
<td>27.08 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>31.14 ± 2 (High T)</td>
<td>31.14 ± 2 (High)</td>
</tr>
<tr>
<td>Salinity</td>
<td>36.0 ± 0.4</td>
<td>36.3 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>36.4 ± 0.72 (High T)</td>
<td>36.6 ± 0.67 (High T)</td>
</tr>
<tr>
<td>$A_T$ (μmol Kg$^{-1}$)</td>
<td>2359.2 ± 83</td>
<td>2344.6 ± 97</td>
</tr>
<tr>
<td></td>
<td>2361.3 ± 93 (High T)</td>
<td>2352.7 ± 104 (High T)</td>
</tr>
<tr>
<td>pCO$_2$ (μatm)</td>
<td>432.5</td>
<td>2023.4</td>
</tr>
<tr>
<td></td>
<td>450.7 (High T)</td>
<td>2154.1 (High T)</td>
</tr>
<tr>
<td>DIC (μmol Kg$^{-1}$)</td>
<td>2043.05</td>
<td>2297.08</td>
</tr>
<tr>
<td></td>
<td>2016.05 (High T)</td>
<td>2293.05 (High T)</td>
</tr>
<tr>
<td>HCO$_3^-$ (μmol Kg$^{-1}$)</td>
<td>1805.77</td>
<td>2172.77</td>
</tr>
<tr>
<td></td>
<td>1759.76 (High T)</td>
<td>2162.92 (High T)</td>
</tr>
<tr>
<td>CO$_3^{2-}$ (μmol Kg$^{-1}$)</td>
<td>225.71</td>
<td>70.28</td>
</tr>
<tr>
<td></td>
<td>245.34 (High T)</td>
<td>77.85 (High T)</td>
</tr>
<tr>
<td>$\Omega$ Aragonite</td>
<td>3.59</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>3.98 (High T)</td>
<td>1.26 (High T)</td>
</tr>
<tr>
<td>$\Omega$ Calcite</td>
<td>5.41</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>5.90 (High T)</td>
<td>1.87 (High T)</td>
</tr>
</tbody>
</table>
Table 2. Results of statistical analyses. All response variables were analyzed using a linear mixed effects model, including all interaction terms, with pH, temperature, and pairing (maternal/paternal pair) as fixed effects and culture tube as a random effect (DF = 98). Significant effects (P < 0.05) shown with an *.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Std Error</th>
<th>t-Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>-37.51</td>
<td>11.76</td>
<td>-3.19</td>
<td>0.00*</td>
</tr>
<tr>
<td>pH</td>
<td>-7.90</td>
<td>11.76</td>
<td>-0.67</td>
<td>0.50</td>
</tr>
<tr>
<td>Pairing</td>
<td>-10.08</td>
<td>3.12</td>
<td>-3.23</td>
<td>0.00*</td>
</tr>
<tr>
<td>Temp:pH</td>
<td>1.85</td>
<td>16.62</td>
<td>0.11</td>
<td>0.91</td>
</tr>
<tr>
<td>Temp:Pairing</td>
<td>3.61</td>
<td>4.41</td>
<td>0.82</td>
<td>0.41</td>
</tr>
<tr>
<td>pH:Pairing</td>
<td>3.57</td>
<td>4.41</td>
<td>0.81</td>
<td>0.42</td>
</tr>
<tr>
<td>Temp:pH:Pairing</td>
<td>-2.09</td>
<td>6.23</td>
<td>-0.34</td>
<td>0.74</td>
</tr>
</tbody>
</table>
FIGURE CAPTIONS

**Figure 1.** Unfertilized Tridacna maxima eggs. Image of newly spawned, unfertilized, T. maxima eggs showing jelly coat surrounding cell cytoplasm.

**Figure 2.** Elevated temperature reduces Tridacna maxima fertilization success. Mean fertilization success (± 95%-CI, error bars) in T. maxima under stressor conditions (Acidified, pH 7.6; High Temp, 31°C; or Multistressor, pH 7.6, 31°C) relative to that of the control group (pH 8.1, 27°C). Mean fertilization success of the control is given in the upper right (shaded region denotes 95%-ci). Letters indicate significant differences between means.

**Figure 3.** Some individuals were particularly sensitive to elevated temperature. Mean fertilization success (± 95%-CI, error bars) in Tridacna maxima under ambient (27°C, open circles) or elevated temperature (31°C, filled circles) plotted against experimental pH conditions (acidified, pH 7.6; or ambient pH 8.1). Letters indicate significant differences between means.
FIGURES

Figure 1.
Figure 2.
Figure 3.
ABSTRACT

Overexploitation of coral reef communities is of growing concern globally. No-take marine reserves are increasingly employed as tools to protect biodiversity and facilitate recovery of exploited populations. In response to declining stocks, the island of Mo’orea, French Polynesia, established a series of eight no-take marine reserves in 2004. Those reserves, the Plan de Gestion de l’Espace Maritime network (PGEM), cover 20% of the island’s fringing reefs. The PGEM was intended to promote recovery of several exploited species, including the threatened giant clam, Tridacna maxima. T. maxima is an economically important species in French Polynesia where it displays conspicuous color morphs that are easily seen by fishers and that are valuable in the aquarium market, and may therefore be particularly subject to overharvesting. I conducted a total of 30 surveys covering 2250 m$^2$ of reef to assess collection-driven alterations of population demography and state of recovery of T. maxima within the Mo’orea PGEM. Despite minimal external enforcement of regulations, clam abundance has increased nearly 3-fold in the PGEM while remaining unchanged outside the reserve. Giant clams were 6x more abundant in protected sites than non-protected controls and densities of mature and conspicuously-colored T. maxima clams were 12x and 4x greater within the PGEM, respectively. These population increases are 1.7x higher than the average reported for other marine reserves globally, and have likely been positively influenced by social factors including traditional Polynesian conservation practices. These results indicate that the PGEM network of Mo’orea is an effective and viable solution for promoting recovery of a threatened giant clam.

INTRODUCTION

Coral reef ecosystems are some of the most diverse habitats on earth and are of major importance to many island communities where they support the development of local and national economies (Dalzell et al. 1996; Moberg and Folke 1999; Ferraris and Cayré 2003; Kronen et al. 2010; Pickering et al. 2011). Reefs, however, are becoming critically threatened by overfishing as a result of overpopulation and overexploitation of commercial fisheries species (Hughes 2003; Pandolfi et al. 2003). A recent global analysis has indicated that 75% of coral reef ecosystems are currently severely threatened by anthropogenic pressure, and this figure is predicted to rise to 90% by 2030 and 100% by 2050 (Burke et al. 2011). Even in remote regions of the South Pacific where human impacts on reefs have been less intense (Vieux et al. 2004), extractive local
economies have resulted in incipient overexploitation of marine resources (Planes et al. 1993; Van Wynsberge et al. 2016). No-take marine reserves have been promoted as effective tools for protecting reef biodiversity, maintaining ecosystem function, and permitting recovery of overexploited species (Balmford et al. 2004; Aburto-Oropeza et al. 2011) and some of the largest marine reserves are located in the archipelagos of the tropical Pacific Ocean (Russ et al. 2008). However, inclusive of the creation of these extensive reserves, less than 3% of the world’s oceans are currently protected from extractive activities (5.7% of coastal regions) and increasing coastal populations are putting further strain on even these remote marine resources (Vieux et al. 2004).

Despite their relative isolation, Pacific Island communities still face many conservation threats. In these regions, fishing pressure is strongly correlated with human population density (Russ and Alcala 1989; Jennings 1998) and increases in fishing pressure have resulted in size-selective changes in target fisheries populations and alterations of reef ecosystem communities (Jennings and Polunin 1996). On the island of Mo’orea (Society Islands Archipelago, French Polynesia), population census figures between 1971 and 2007 show annual population growth rate of 2.4%, which is higher than the rate for French Polynesia as a whole (1.6%) (Leenhardt et al. 2016). Effects of overfishing in Mo’orea were first noticed in 1991, when local citizens and scientists observed that both the sizes and overall catches of fish in Mo’orea’s lagoon were declining (Lison de Loma 2005; Walker and Robinson 2009). Concern over this decline led to the implementation of a comprehensive marine management plan, the Plan de Gestion de l’Espace Maritime (PGEM), to protect impacted marine resources (Salvat and Aubanel 2002). Established in 2004 with the input of local and extra-territorial stakeholders, the PGEM designated a network of eight no-take marine reserves covering approximately 20% of Mo’orea’s lagoon and established size and catch limits on commercial fish and mollusc species throughout the remaining non-protected areas of the lagoon (Lison de Loma et al. 2008; Walker and Robinson 2009). Among those species designated for special protection was the giant clam, Tridacna maxima.

Giant clams are the largest living bivalves and are found throughout the tropical Indo-Pacific where they have ecological and cultural economic importance (Neo et al. 2015; Van Wynsberge et al. 2016). In many developing island territories, including French Polynesia, giant clams are harvested for seafood, and for the ornamental shell and aquarium hobbyist trades (Pickering et al. 2011). In these regions, giant clam products can rank as the number one national export with annual export production values exceeding $90 million USD or ~5% of total Gross Domestic Product (Solomon Islands, 2011 UN World Development Indicator GDP estimates). While giant clams can be numerous on coral reefs (Gilbert et al. 2006) and are capable of mass recruitments (Braley 1988), their large size and slow maturation (12-13 years on average; Mies et al., 2012) make them susceptible to overexploitation (Lucas 1994; Neo et al. 2015). Across much of their range, intensive harvesting has depleted wild stocks (Guest et al. 2008; Neo and Todd 2013). As a result, all species of giant clams are now listed on the Convention on International Trade in Endangered Species (CITES) as species of concern, vulnerable to local extirpations (Lucas et al. 1989; Planes et al. 1993; Gilbert et al. 2006; Pickering et al. 2011; Neo and Todd 2013).

Localized extirpations of giant clams have been recorded over the past several decades. For example, Tridacna gigas (the largest giant clam species) and Hippopus hippopus were once
present on Singapore’s reefs, but recent surveys conducted in 2003 and 2009/10 (and covering 9670 m$^2$ and 87515 m$^2$ of reef respectively) failed to find a single individual of either species (Guest et al. 2008; Neo and Todd 2013). Similarly, the previously common T. maxima was also nearly absent; only a single individual was found in 2009/10 suggesting that this once abundant bivalve is now functionally extinct over much of Singapore’s reefs (Neo and Todd 2013). At another site in the Lakshadweep Archipelago of India, populations of T. maxima declined precipitously – decreasing by up to 33% in just one year (2005-2006) – as a result of increasing harvesting pressure following rapid human population growth in nearby coastal communities (Apte et al. 2010). While giant clam population densities remain comparatively high in the more remote and less intensively exploited regions of the Pacific (e.g. the Tuamotu Archipelago of French Polynesia where clam densities are an order of magnitude higher than at more populated reefs, (Gilbert et al. 2005, 2006; Van Wynsberge et al. 2016)), recent surveys have shown signs of incipient overexploitation of even these isolated populations. In French Polynesia, surveys in the early 1990’s of clam stocks in the lagoon of Bora Bora showed that tourism-related harvesting of T. maxima had resulted in a reduction in mean individual size of 23% over the course of a year, indicating rapid collection-induced alteration of demography in this population (Planes et al. 1993). To avoid similar declines in the abundance of T. maxima on the island of Mo’orea, the PGEM established both no-take zones and strict size restrictions on giant clam collection across the remaining reef (Lison de Loma 2005).

The regulations regarding T. maxima collection on Mo’orea are straightforward: within PGEM sites, collection of T. maxima is strictly forbidden, whereas outside of protected sites, only clams with a shell length of > 10 cm may be harvested. However, catch restrictions on Mo’orea remain largely unenforced and therefore the protective benefits of the PGEM network depend on voluntary local compliance. Furthermore, though the no-take reserves of the Mo’orea PGEM may protect giant clams within designated protected zones, fishing pressure could be intensified at non-protected sites, spatially concentrating collection efforts and potentially decimating populations outside the PGEM network. In addition, current PGEM catch-size regulations permit harvesting only of reproductively mature adult clams (>10 cm shell length; DIREN Polynésie française, 2005), potentially reducing reproductive potential of giant clam populations on Mo’orea’s reefs. Focused harvesting of only mature adults has been shown to result in rapid shifts in population structure in giant clams populations of the Lakshadweep Archipelago, reducing both the number and size of reproductively mature individuals (Apte et al. 2010). Thus, the current regulatory framework of the PGEM might be expected to result in severe negative impacts for giant clams inhabiting non-protected sites, leading to drastic reductions in adult broodstock and potentially impeding larval replenishment across Mo’orea’s reefs. Further, the iridescent coloration of giant clams’ mantles that make them so desirable as aquarium specimens also makes them particularly conspicuous on the reef (Fig. 1) and might in turn result in a disproportionate removal of brightly-colored clams from non-protected sites. Whereas population monitoring surveys have been conducted across Mo’orea’s reefs since 2004 (Lison de Loma et al. 2008) to report potential declines in stock, this monitoring plan does not address possible reductions in mean individual size or age and potential loss of highly conspicuous (and economically valuable) color-phenotypes.

To investigate whether the PGEM network has effectively protected giant clams and shifted demographics of populations outside protected sites, I conducted surveys to assess abundance
and population demography of the giant clam, Tridacna maxima, on the fringing reefs of Mo’orea, French Polynesia. I quantified current population structure of T. maxima relative to that reported prior to PGEM establishment, across a series of paired PGEM and non-protected control sites to address hypotheses regarding exploitation-driven size- and color-selection of giant clams outside of Mo’orea’s established PGEM, namely whether clams are more abundant, larger, and more brightly-colored in protected regions versus on nearby non-protected reefs. I also report on demographic trends over the ca. 12-year period spanning PGEM establishment to assess giant clam population recovery rates on Mo’orea’s fringing reef within and without the marine reserve.

**METHODS**

**Site Selection and Field Surveys**

Field surveys were carried out at seven locations on fringing reefs of the island of Mo’orea in the Society Islands Archipelago of French Polynesia (Fig. 2, Table 1). At six of the seven locations, two paired sites were surveyed, one within a protected region (PGEM site) and one at a nearby unprotected section of the reef (control site). Only one location (Site 7, Haapiti; Fig. 2) lacked a paired PGEM survey site. Controls sites were located at least 50 m away from the PGEM border (as demarcated by official notification posts installed on the reef during PGEM designation) in order to reduce edge effects in survey estimates.

A total of 29 surveys were conducted across these seven locations covering a total reef area of 1450 m² (Table 1). All surveys were conducted in 25 m x 3 m belt transects arranged parallel to shore along the fringing reef, 3-15 meters inshore from the reef crest. Surveys were conducted between 9h00 and 17h30 in 1-3 meters of water during September and October of 2015 and 2016. The number, size (shell length), and predominant coloration of the mantle (teal, blue, brown; Fig. 1) of all T. maxima clams within each quadrat were recorded.

**Population Recovery Assessment**

Historical data on T. maxima abundance prior to PGEM establishment were collected from two repositories: unpublished survey data from 2001 conducted at one location (two paired sites) at the UC Berkeley Richard Gump Field Station (Wagner 2001) and a dataset of annual benthic invertebrate surveys (suivi des invertébrés benthiques AMP) available from the Centre de Recherches Insulaire et Observatoire de l’Environnement (CRIOBE) Service d’Observation CORAIL (SO CORAIL) conducted at three locations (six paired sites) over the period from 2004 to 2016 across Mo’orea’s MPAs (Galzin et al. 2006).

UC Berkeley Surveys in 2001 were conducted during October/November at six locations on Mo’orea using belt transects (30 x 3 m) running parallel to shore and carried out at 1-3 m depth (Wagner 2001). Four of these sites were also resurveyed in this study: Hilton, Pihaena, Temae,
and Haapiti (Fig. 2). Although it was not possible to replicate the exact survey corridors utilized in 2001, belt transects were conducted as close as possible to original survey locations on sections of fringing reef at similar depth. SO CORAIL surveys were conducted in a similar manner to those performed in this study save that no information on clam coloration or size was available from the historical data (Galzin et al. 2006).

**Data Analysis**

The effects of the PGEM network on each response variable (i.e. giant clam abundance, and size- and color-distribution) were examined independently using Paired t-Tests in the statistical software program R (v 3.3.2). Historical trends were analyzed using a Before-After-Control-Impact Series assessment as described by Lison de Loma et al. (Lison de Loma et al. 2008). Briefly, changes in clam density within the PGEM between pre- and post-reserve establishment surveys were compared to those occurring over the same period in non-protected controls using a Two-Tailed Paired t-Tests, a conservative estimate given the likely positive effect of PGEM establishment on clam population density (Lison de Loma et al. 2008).

**RESULTS**

**Population Demography**

Clams were not evenly distributed across Mo’orea’s fringing reefs; higher densities of clams were observed on the northeastern and eastern shores of the island relative to the west and south, where clams were very sparse (Fig. 2). In addition, there were significant differences in T. maxima population structure between protected and non-protected sites (Table 1). Density of juvenile clams within PGEM sites (0.49 ± 0.11 ind. m$^{-2}$; $\bar{x}$ ± se) was significantly greater than in non-protected sites (0.12 ± 0.03 ind. m$^{-2}$; Paired Two-Tailed t-Test, df = 12, p < 0.05; Fig. 3A). Likewise, density of mature clams within PGEM sites (0.12 ± 0.03 ind. m$^{-2}$) was also significantly greater than in non-protected sites (0.01 ± 0.00 ind. m$^{-2}$; Paired Two-Tailed t-Test, df = 12, p < 0.01; Fig. 3A). Overall, clams were 600% abundant within PGEM sites (0.30 ± 0.07 ind. m$^{-2}$) than paired controls (0.05 ± 0.03 ind. m$^{-2}$).

Conspicuously-colored blue and teal clams were significantly more abundant within the PGEM network (Fig. 3B). Collectively, conspicuously-colored clams were found at a density of 0.004 ± 0.001 ind. m$^{-2}$ within PGEM sties as compared to 0.001 ± 0.000 ind. m$^{-2}$ in controls (Paired Two-Tailed t-Test, df = 14, p < 0.05; Fig. 3B). Individually, blue and teal clams were found to be ca. 400% and 550% more abundant, respectively, than in paired non-protected sites. However, despite being more abundant within PGEM sites, conspicuously-colored clams were not over-proportionally represented in protected area populations. Clams with blue and teal mantles comprised 12.97 ± 8.34% ($\bar{x}$ ± 95%-CI) and 5.40 ± 2.89% of the population respectively in PGEM sites as compared to 4.20 ± 4.58% and 2.68 ± 3.02% as observed in control locations (Paired t-Test, df = 14, p > 0.1 and p > 0.05, respectively).
Recovery Estimates

Comparison of time-normalized population density trends from before and after PGEM establishment across all locations (one UC Berkeley and three SO CORAIL site pairs) indicates that T. maxima density has increased significantly at PGEM sites relative to non-protected controls: +22.0 ± 9.0% yr\(^{-1}\) (\(\bar{x} \pm \text{se}\)) versus -0.1 ± 3.2% yr\(^{-1}\) respectively (Paired Two-Tailed t-Test, \(t = 3.13, \text{df} = 3, p = 0.05\); Fig. 4A). Within the three SO CORAIL survey locations this resulted in a significant 207% increase in giant clam population density in the PGEM (from an average of 0.053 ± 0.01 ind. m\(^{-2}\) recorded in 2004 (Galzin et al. 2006) to 0.14 ± 0.04 ind. m\(^{-2}\) in 2016) as compared to a 57% decrease in paired control sites (from 0.093 ± 0.01 ind. m\(^{-2}\) to only 0.053 ± 0.01 ind. m\(^{-2}\) over the same time period; Figure 4B). Similarly, average clam density within the UC Berkeley PGEM focal site (Pihaena) increased from 0.12 ind. m\(^{-2}\) in 2001 to 0.89 ind. m\(^{-2}\) in 2016. This represents a nearly 460% increase in clam abundance relative to the paired control site wherein clam density was measured at 0.08 ind. m\(^{-2}\) and 0.19 ind. m\(^{-2}\) in 2001 and 2016 respectively (Figure 4B inset; Wagner, 2001).

DISCUSSION

In this study, I sought to address whether the PGEM no-take marine reserve network is an effective tool for preserving giant clam population density and phenotypic diversity on Mo’orea’s reefs. I demonstrate that the Mo’orea PGEM has been an exceptionally effective reserve for promoting recovery of the threatened giant clam, Tridacna maxima. Greater clam density and larger clam size within PGEM sites relative has resulted in a significant increase in average clam biomass relative to non-protected controls. In addition, over its 12-years of establishment, the PGEM network has seen an increase in clam population density, in stark contrast to a significant decrease observed over this same period across control sites (Fig. 4B).

The PGEM Network Supports Higher Densities of T. maxima on Mo’orea’s Reefs

Tridacna maxima was significantly more abundant within PGEM sites being on average 6-times more abundant than in nearby non-protected control regions. While conspicuously-colored clams were not found to be disproportionately absent from non-protected sites, they were significantly more abundant with the PGEM network (Fig. 3B). This finding refutes the hypothesis that brightly-colored clams are selectively removed from non-protected sites. However, PGEM sites do host significantly greater numbers of mature individuals (Fig. 3A). Within the 2250 m\(^2\) of reef surveyed, only 58 adult clams were encountered outside of the PGEM network with 57 of these found at a single, highly populated location, the Ahi-Popaa reserve pair (location 3; Fig. 2). This striking imbalance is not surprising given the PGEM catch restrictions – only clams > 10 cm (i.e. mature adults) can be collected at non-protected sites – but poses significant ecological consequences for Mo’orea’s T. maxima populations.

Giant clams are iteroparous, reproducing repeatedly over their long lifespans, and thus employ a “bet-hedging” technique which may result in infrequent, but abundant recruitment to the
environment (Braley 1988; Roberts 2005). In addition, the planktonic larvae of giant clams leave open the possibility for recruitment of individuals into non-protected sites, thus permitting “spill over” effects from marine reserves to nearby or even potentially distant reefs. However, for many giant clam species, successful recruitment episodes are tightly linked to favorable oceanographic conditions which occur infrequently or sporadically and may limit population recovery even despite frequent reproductive events (Louis W. Botsford et al. 1997; Roberts 2005). Thus, the continued presence and productivity of a healthy population of mature broodstock may be critical for maintaining a viable population of giant clams. The scarcity of mature individuals at non-PGEM sites therefore represents a significant ecological hurdle to reestablishment of T. maxima at these locations. Unless larvae are recruited to these sites from reproductive episodes occurring in the PGEM, giant clam populations cannot increase beyond current estimates over much of Mo’orea’s fringing reef. This makes the T. maxima populations of Mo’orea an ideal system for further study of the magnitude and efficacy of “spill-over” effects from no-take marine reserves.

The PGEM Network is an Exceptionally Effective Marine Management Area

Over the ca. 12-year period examined in this study, T. maxima populations have increased approximately 3-fold in Mo’orea’s PGEM sites relative to non-protected controls. This is particularly striking when compared with the general decrease in population observed over the same time period (-6%) in non-protected sites. Marine reserves can facilitate such rapid population recoveries, and doubling or even tripling of stocks within five years of protection is not uncommon (Castilla and Durán 1985; Roberts 1995; Wantiez et al. 1997; Roberts et al. 2001). While T. maxima has taken longer to reach a similar recovery state (12-years versus 5), this is likely accounted for by its slow growth relative to many other commercially harvested species.

In a meta-analysis of 89 MPA studies, Halpern found that MPA establishment increases biomass of protected species by 192% on average (Halpern 2003). This result is considerably higher than that of a subsequent meta-analysis of 218 MPAs which concluded that in 71% of cases, MPAs yielded positive ecosystem effects including an average biomass increase of ca. 60% (Gill et al. 2017). This later meta-analysis also demonstrated that the majority of positive outcomes are restricted to protected areas with adequate staffing and enforcement (Gill et al. 2017). In reserves with minimal or no enforcement, species recovery was significantly impeded. Unenforced, multi-use MPAs displayed very little change, or even slight declines in average biomass, on the order of -1% (Gill et al. 2017). Mo’orea’s PGEM network stands in stark contrast to this general trend (Fig. 5); despite minimal enforcement, the protected site network has permitted above average recovery of an exploited species when compared to the global average. Whereas unenforced marine reserves typically effect biomass increases on the order of +125% relative to similar, unprotected, habitats, T. maxima populations within PGEM sites have shown a significantly greater rate of increase (+322%) which is on par with recovery rate seen in adequately staffed and enforced marine reserves (Fig. 5). Furthermore, the relatively high recovery rate demonstrated in T. maxima has also been observed in other fisheries species in Mo’orea (e.g. parrotfishes, Adam et al., 2011) suggesting that, while the precise magnitude of population rebound may be taxon specific, there is strong support for the PGEM as a uniquely effective resource management tool in French Polynesia.

49
One potential explanation for the relative success of the PGEM network is the conscious inclusion of local stakeholders in its design and implementation. Previous research has shown that marine reserves can be especially effective at promoting species recovery when local communities support their implementation and regulation and are actively engaged in self-enforcement (Aburto-Oropeza et al. 2011; Vance 2017). In French Polynesia, these effects are potentially amplified by an explicit recognition and incorporation of a traditional Polynesian conservation practice – the rahui. Because of their traditional reliance on limited resources of isolated island systems, Polynesian culture has a rich preservation ethic and has traditionally enforced ecologically conservative practices including restricted access to those resources considered sacred (Ghasarian 2016). In addition, the relative isolation of many of French Polynesia’s communities results in a lack of free market competition for resources and thus incentivizes following conservation regulations (Thorax 2016). Furthermore, besides their emerging value as an economic export, giant clams (Tahitian pahua) are also culturally important to the Polynesian people who use them for food and in ritual ceremonies. Giant clams also feature prominently in several formative tales of the Polynesian oral tradition (e.g. the travels of the Voyager King Rata (Weckler Jr 1943; Firdausy and Tisdell 1992)) and are an important part of Islanders’ ethnic cultural identity. Indeed, one of the driving factors behind implementation of Mo’orea’s PGEM was a local concern for preserving several of these culturally significant species including giant clams (Salvat and Aubanel 2002; Lison de Loma et al. 2008). Given the relative success of the PGEM for preserving these species, further research into local perceptions of the resource management network, especially in regards to cultural or ideological impacts, is certainly warranted.

Despite the apparent success of the PGEM network in facilitating the recovery of giant clam stocks on Mo’orea, caution should still be taken in capitalizing on this success and opening reserves for harvesting. In another long-lived mollusc, the red abalone (Haliotis rufescens), opening of a no-take reserve to fishing resulted in a nearly 70% decline in population in just three years (Rogers-Bennett et al. 2013). In addition, fishing resulted in a shift in the size frequency distribution of abalone towards smaller individuals which in turn lead to a greater than 72% decrease in the egg production potential of the population over the same short time interval (Rogers-Bennett et al. 2013). This decreased reproductive potential likely already impacts a significant portion of Mo’orea’s fringing reefs where mature adult clams are entirely absent in open harvest areas. While the no-take PGEM has proven especially effective in restoring and maintaining a viable T. maxima population on Mo’orea its efficacy is ultimately dependent on the sanctuary the protected reserves provide to reproductively mature broodstock clams. Given the slow development of T. maxima and its demonstrated susceptibility to even minimal local demand (Planes et al. 1993), it is likely that persistence of giant clam populations on Mo’orea will require continued adherence to the catch restrictions currently instituted under the PGEM.

ACKNOWLEDGEMENTS

I would like to thank Camilla Souto, Arthur, Barbara, and Brittany Armstrong, and Amanda Horvath for their invaluable assistance in field surveys. Their contagious positivity and continuing encouragement made field expeditions pleasant, even in the most terrific of deluges.
Additional thanks to Drs. Serge Planes and Rène Galzin and the technicians and staff at CRIOBE, especially Franck Lerouvreur and Pascal Ung, for their assistance and support during time spent at CRIOBE and in accessing the SO CORAIL demography data. This research was conducted with US Government support to EJ Armstrong under and awarded by the Department of Defense, Air Force Office of Scientific Research, National Defense Science and Engineering Graduate (NDSEG) Fellowship, 32 CFR 168a.
REFERENCES


Marines Protégées de Moorea.


Walker BLE, Robinson M (2009) Economic development, marine protected areas and gendered access to fishing resources in a Polynesian lagoon. Gender, Place Cult 16:467–484. doi: 10.1080/09663690903003983


Weckler Jr JE (1943) Polynesians: Explorers of the Pacific. Smithsonian Institution, Baltimore, MD, USA
### Table 1. Tridacna maxima Survey Data

Presented are density data (clams m\(^{-2}\)) for each survey site pair (PGEM or non-protected control) including approximate location of surveys, number of replicate surveys (N), and the mean population densities (number of individuals m\(^{-2}\) ± s.d.) of juvenile, adult, and conspicuously-colored T. maxima at each location.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type</th>
<th>Location</th>
<th>N</th>
<th>Juveniles</th>
<th>Adults</th>
<th>Conspicuous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hilton</td>
<td>Control</td>
<td>17º 28’ 48” S, 149º 49’ 48” W</td>
<td>3</td>
<td>0.06 ± 0.11</td>
<td>0</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Pihaena</td>
<td>PGEM</td>
<td>17º 29’ 24” S, 149º 49’ 48” W</td>
<td>3</td>
<td>0.57 ± 0.39</td>
<td>0.12 ± 0.07</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Faaupo</td>
<td>Control</td>
<td>17º 29’ 24” S, 149º 45’ 00” W</td>
<td>2</td>
<td>0.05 ± 0.06</td>
<td>0</td>
<td>0.00 ± 0.01</td>
</tr>
<tr>
<td>Nuarei</td>
<td>PGEM</td>
<td>17º 30’ 00” S, 149º 49’ 48” W</td>
<td>2</td>
<td>1.14 ± 0.10</td>
<td>0.06 ± 0.01</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>Popaa</td>
<td>Control</td>
<td>17º 31’ 48” S, 149º 46’ 12” W</td>
<td>3</td>
<td>0.39 ± 0.42</td>
<td>0.03 ± 0.02</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Ahi</td>
<td>PGEM</td>
<td>17º 31’ 59” S, 149º 45’ 58” W</td>
<td>3</td>
<td>0.49 ± 0.21</td>
<td>0.25 ± 0.18</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Haumi</td>
<td>Control</td>
<td>17º 34’ 34” S, 149º 47’ 49” W</td>
<td>1</td>
<td>0.04</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maatea</td>
<td>PGEM</td>
<td>17º 34’ 51” S, 149º 47’ 45” W</td>
<td>1</td>
<td>0.03</td>
<td>0</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Hauru</td>
<td>Control</td>
<td>17º 30’ 18” S, 149º 55’ 05” W</td>
<td>1</td>
<td>0.07</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetaiuto</td>
<td>PGEM</td>
<td>17º 30’ 29” S, 149º 55’ 01” W</td>
<td>1</td>
<td>0.16</td>
<td>0.01</td>
<td>0.04 ± 0.06</td>
</tr>
<tr>
<td>Club Med</td>
<td>Control</td>
<td>17º 29’ 42” S, 149º 54’ 50” W</td>
<td>3</td>
<td>0.03 ± 0.03</td>
<td>0.00 ± 0.01</td>
<td>0</td>
</tr>
<tr>
<td>Tiahura</td>
<td>PGEM</td>
<td>17º 29’ 17” S, 149º 54’ 54” W</td>
<td>3</td>
<td>0.23 ± 0.05</td>
<td>0.09 ± 0.06</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Haapiti</td>
<td>Control</td>
<td>17º 33’ 00” S, 149º 52’ 48” W</td>
<td>3</td>
<td>0.01 ± 0.01</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
FIGURE CAPTIONS

**Figure 1.** Qualitative mantle color morphs of Tridacna maxima on Mo’orea, French Polynesia. Conspicuously-colored teal (lower left), and blue (upper) clams as compared to the more common, inconspicuous brown (mid-right) mantle patterning.

**Figure 2.** Tridacna maxima shows unequal distribution across locations. Size and color distribution of immature and adult (> 12 cm shell length) Tridacna maxima across survey sites on Mo’orea, French Polynesia (filled and open map circles). Data are plotted as mean clam size with standard error (error bars) at each pair of sites (PGEM reserves shaded). Accompanying numbers denote mean number of individuals per survey. Colors represent qualitative categorization of mantle pigmentation (teal, blue, and brown).

**Figure 3.** Tridacna maxima is more abundant in PGEM sites. (A) Tridacna maxima population density (clams per m²) as measured in 2016 within the seven survey locations for both (A) juvenile and mature clams (> 12 cm shell length), and (B) clams with conspicuously and inconspicuously colored mantles (teal and blue and brown respectively); PGEM sites (filled circle) and their corresponding controls (open circle). Error bars represent standard error around the mean.

**Figure 4.** Giant clam populations have been increasing within the PGEM. (A) Annual percentage change in the number of Tridacna maxima clams per m² at eight paired sites across Mo’orea; four protected PGEM sites (filled circle) and their corresponding controls (open circle). (B) Cumulative percentage change in T. maxima population density over the 12-year SO CORAIL survey period (2004-2016; five sites) and 15-year UC Berkeley survey period (inset; one site). Data are from this study, Wagner 2001, and Galzin et al. 2006. Error bars represent standard error around the mean.

**Figure 5.** The Mo’orea PGEM outperforms other no-take reserves with similarly staffing capacity. Mean change in biomass with standard deviation (error bars) for multi-use sites (open circles), no-take reserves (closed circles), and the Mo’orea PGEM no-take network (triangle) group by reported staffing capacity (none, inadequate, or adequate). Positive values signify biomass increase within protected area relative to paired controls and dotted-line denotes global average biomass response (+192%) as determined by Halpern 2003. Values in parentheses on the y axis denote the number of protected sites that are multi-use and those that are designated no-take reserves, respectively. Data and figure modified from Gill et. al. 2017.
FIGURES

Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.