UNIVERSITY OF CALIFORNIA, MERCED

The ecological and evolutionary effects of environmental perturbations on populations and communities

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

in

Quantitative and Systems Biology

by

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2016

DEDICATION

To my Apodidae family and my little Artemisia
"The mountains are calling…"
All my love

EPIGRAPH

The brook would lose its song if we removed all the rocks
Wallace Stegner

The spaces between the stars are where the work of the universe is done
Ivan Doig

I must go down to the seas again, for the call of the running tide
Is a wild call and a clear call that may not be denied…
John Mansefield, Sea Fever

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Much of science is performed in relative solitude from the larger scientific community until the brief moment the research on which one has toiled is shared with the world. Scientists spend most of their time in these long periods of solitude 'darkness,' punctuated briefly by the 'flashes of light.' You all are my lantern in the space between the stars; thank you.

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ABSTRACT

Environmental perturbations can provide ideal situations to study eco-evolutionary dynamics (feedback loops between ecological and evolutionary interactions) on short timescales. Yet, studies of these dynamics in marine pelagic species, whose populations are not clearly defined, risk obscuring causal relationships. Studies of eco-evolutionary relationships in terrestrial and freshwater systems benefit from utilizing microcosms, which can act as 'natural laboratories' to allow better measures of the acting processes. Yet, only recently have marine systems been recognized to have analogous microcosms. I utilized one such marine microcosm – marine lakes – to study a contained population of a pelagic scyphozoan predator, *Mastigias papua.* Across the Indo-West Pacific, *Mastigias* spp. occurs in two distinct morphological ecotypes – ocean and lake – that occur locally proximate to one another, yet appear similar within ecotype across the region. I established the evolutionary history, locally and regionally, by reconstructing phylogenetic and intraspecific genetic relationships utilizing two nuclear (H3a, H3b) and three mitochondrial (COI, COIII, 16S) markers. Phylogenetic reconstruction was complemented by morphological characters to reconstruct the macroevolution of ecotypes and test three hypotheses that explain the geographic distribution of ecotypes. Periodic perturbations, consisting at least in part of a highly stratified layer of warmer and more saline water and the disappearance of the medusae stage of *Mastigias*, occur in the marine lakes. I examined the microevolutionary effects of one such event on the genetics, morphology and behavior in one Palauan lake population. I used the genetic data to exclude one possible hypothesis that explained the difference in morphology and behavior upon medusae return than before the perturbation event. Finally, I examined the associations between environmental factors and *Mastigias* medusa population size following another such perturbation in a different Palauan marine lake over a decade, as well as examined the associations between medusae and other microplankton population fluctuations. These studies build a foundation for eco-evolutionary work in marine systems. The comparative framework across spatial and temporal scales can elucidate patterns and processes that act in the marine realm. As climatic variability increases, it is increasingly important to understand the eco-evolutionary dynamic of systems and the potential impacts perturbations can have on those systems.

CHAPTER 1:

INTRODUCTION – APPLYING ECO-EVOLUTIONARY DYNAMICS IN THE PELAGIC REALM

1 Context

The iterative effect of ecological change on evolution was first seen in Hutchinson's ecological theater (1965) and has long been a focus of scientific research. Only recently has the reverse – how evolutionary dynamics affect ecological traits – garnered attention with the realization that evolution can occur over ecological time scales (Schoener 2011). The major precept of eco-evolutionary dynamics is that both directions of effect – environment to ecology to evolution and vice versa – are important. Additionally, the rapid evolution of species and their interactions can impact community ecology (Thompson 1998, Hairston et al. 1999, Thompson et al. 2001), although investigations of these processes in natural ecosystems are scarce (Orr & Smith 1998). Most studies have focused unidirectionally on effects of ecological change on evolution or constructed a framework from multiple component studies. Thus, the relative importance and influence of how evolutionary dynamics affect ecological traits in populations, species, and communities is still largely unknown (Thompson et al. 2001, Schoener 2011). Untangling the dynamic requires long-term field experiments to address each of its components (Thompson et al. 2001, Schoener 2011).

Environmental perturbations, sudden shifts in the 'normal' environmental state, can provide an ideal situation to study eco-evolutionary dynamics on short timescales. From an eco-evolutionary perspective, environmental perturbations can shift genotype frequencies (Grant & Grant 2002, Brierley & Kingsford 2009, Lamichhaney et al. 2015), change community assemblages (Klug et al. 2000), cause (plastic) morphological or physiological changes in species (Boag & Grant 1981, Argasinski & Broom 2013), or potentially shift populations to an alternative state (Schluter 2000). Although potentially short in duration, environmental perturbations can have large impacts on organisms, communities, and ecosystems (Jentsch et al. 2007). These effects are difficult to observe and quantify in natural systems, making the scale, duration and permanence of effects largely unknown (Jentsch et al. 2007). As climate variability increases (IPCC 2007, Jentsch et al. 2007, Kharin et al. 2007), the impact that perturbations can have on systems becomes increasingly important to understand.

Understanding the processes that influence evolution and ecology can be difficult to tease apart in many communities and ecosystems (Thompson et al. 2001). Microcosms – simplified versions of a more complex system (MacArthur & Wilson 1967) – can act as 'natural laboratories' to better measure the relative influences and processes acting within the system. Thus, the clearest examples of evolutionary processes have come from clearly delineated, island systems (Baldwin et al. 1990, Meyer 1993, Schluter & Nagel 1995, Losos et al. 1998, Seehausen 2006, Grant & Grant 2009, Losos 2009). Island populations

have heightened evolutionary forces (Losos & Ricklefs 2009) from reduced gene flow (Petren et al. 2005), founder effects (Losos et al. 1997), smaller population sizes (Grant et al. 2001), novel or open niches (Schluter 2000) and shifting local optima (Ackerly 2003). There are many examples of radiations into new environments causing expansion into new niches, commensurate with morphological and then genetic evolution (finches: Grant 1981, cichlids: Rüber et al. 1999, Hulsey et al. 2013, sticklebacks: Schluter & Nagel 1995, Rundle et al. 2000). In each case, the morphological evolution which occurs creates ecological consequences in shifting niches and trophic interactions (Boag & Grant 1981, Schluter & Nagel 1995, Magalhaes et al. 2009). For these reasons, organisms on islands often exhibit distinct evolutionary changes on ecological time-scales (Boag & Grant 1981, Loso et al. 1997, Losos & Ricklefs 2009). While understanding of the ecological and evolutionary patterns and processes in terrestrial and freshwater systems have increased from studies utilizing microcosms, marine systems have only recently been recognized to have parallel 'island' microcosms in which pelagic organisms occur (Dawson 2015, Dawson et al. 2016).

Marine lakes – small bodies of seawater entirely surrounded by land – provide a rare opportunity for studying the eco-evolutionary dynamics of marine systems in a microcosm. Local topography, lake bathymetry, sedimentation rates and sediment cores analyzed for organic geochemical proxies and dated using ^{14}C indicate marine lakes formed after the Last Glacial Maximum when rising sea levels flooded through cracks of karst islands (Dawson et al. 2009, Smittenberg et al. 2011) into low-lying areas 100 – 260 m from the ocean (Dawson & Hamner 2005). Marine lakes vary in their connection to the ocean; all have tidal cycles, but the timing and amplitude can vary from that of the ocean depending on the degree of connectivity (Hamner & Hamner 1998). Some lakes are, at least, genetically isolated from the ocean; each harbors phenotypically and genetically distinct populations and communities. Additionally, large environmental perturbations have occurred in at least one marine lake in Palau, resulting in the dramatic population size decline in the rhizostome medusae, *Mastigias papua* (Dawson et al. 2001, Martin et al. 2006). The rapid evolution of organisms within the marine lakes, coupled with the extant ocean populations and the varying levels of connectivity of peripheral populations, makes marine lakes an ideal 'natural laboratory' (Dawson & Hamner 2005), especially to study parts of the eco-evolutionary dynamic continuum in populations and communities following environmental perturbations.

Mastigias papua is a rhizostome jellyfish (Class Scyphozoa) that occurs in the Indo-West Pacific (Kramp 1961) in coastal waters including semi-protected coves and in marine lakes (Dawson et al. 2009). Lake ecotypes are morphologically distinct from the cove population (Dawson 2005b, Dawson & Hamner 2005) and visually striking (Fig. 1). Additionally, daily horizontal migrations by *Mastigias* medusae have evolved in some lakes, changing from the west-to-east pattern seen in coves to several variations in the lakes, including a complete reversal to an east-to-west pattern in one lake in response to predator selection pressure (Hamner & Hauri 1981, Dawson & Hamner 2003). The documented morphological and behavioral evolution of *M. papua* could influence other traits and the interactions of *Mastigias* in the community.

Understanding eco-evolutionary dynamics in a system involves the interplay of many different levels of organization and scales (Fig. 2) and thus requires clearly defined

evolutionary and ecological scaffolding, namely, 1) a framework of evolution, and 2) a defined population. Evolutionary frameworks could consist of sister species, members of an adaptive radiation (e.g. Galápagos finches), synchronously diverging co-distributed species (i.e. Dawson 2014), and/or 'natural laboratories.' Defined populations are important since evolution, on eco-evolutionary time-scales, is acting on populations; thus, analyses at a larger scale have the potential to mask causes and consequences, obscuring the eco-evolutionary relationship. Only when these two components are met do clear dynamics emerge, without confounding influences that mask causal relationships. *Mastigias* populations within marine lakes offer the rare opportunity to study contained populations of a pelagic scyphozoan predator. Scyphozoan populations are difficult to study through time, since the medusa and polyp stages can be separated geographically as the pelagic medusae are advected with currents. Thus, marine lakes provide a unique opportunity to study aspects of the eco-evolutionary continuum for an important pelagic predator (Purcell 2005). Eco-evolutionary dynamics simply emphasize that evolution can influence ecology as much as the reverse (Schoener 2011). Although ideally studies would describe the entire eco-evolutionary dynamic, the reality is that our knowledge is best for systems in which long-term studies have produced incrementally more complete information (e.g. Galápagos finches). Building up a scientific body of knowledge, even piece-by-piece, is important as environmental perturbations become increasing prevalent, potentially increasing eco-evolutionary forces.

2 Dissertation Objectives

My dissertation has started to build up that body of knowledge for the scyphozoan *Mastigias papua.* I do so by first establishing the framework of evolution for *Mastigias* spp. across their range in the Indo-West Pacific, and then by examining single populations for ecological or evolutionary responses to extreme perturbation events. Chapter 2 focuses on the evolutionary patterns and relationships that underlie the two distinct morphological ecotypes in *Mastigias* spp. and the morphological crypsis within each. Phylogenetic relationships are reconstructed using three mitochondrial (COI, COIII and 16S rDNA) and two nuclear (H3a and H3b) markers and the tips of one clade further resolved with an intraspecific haplotype network (COI and COIII) that employs finer-scale sampling than the phylogenetic analysis. Morphological characters are mapped onto the reconstructed phylogeny to assess three hypotheses that would explain the geographic distribution of *Mastigias* morphological ecotypes. Chapter 3 analyzes variation of the lake ecotype within one lake, Ngermeuangel Lake, Palau in response to an extreme environmental and density perturbation and the evolutionary sources of the variation. Morphological and behavioral variation is compared between pre- and post-perturbation demes, and genetic variation is assessed to distinguish between hypotheses that would explain the ecophenotypic variation. Chapter 4 builds upon the investigations of the previous chapter, extending the temporal scale to assess population abundance and associated community responses over a decade following a similar perturbation in Ongeim'l Tketau, Palau. These studies, together, provide an initial assessment of key components of the eco-evolutionary dynamics in a scyphozoan.

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4 Figures

Figure 1. Typical morphologies of *Mastigias papua* medusae (from Dawson & Hamner 2005) in the ancestral cove and derived marine lake forms in Palau. Medusae from (left to right) Ngermeuangel Cove, Koror (NCK), Uet era Ongael, Ongael (OLO), Goby Lake, Koror (GLK), Ongeim'l Tketau, Mecherchar (OTM), Clear Lake, Mecherchar (CLM), and Uet era Ngermeuangel Lake, Koror (NLK).

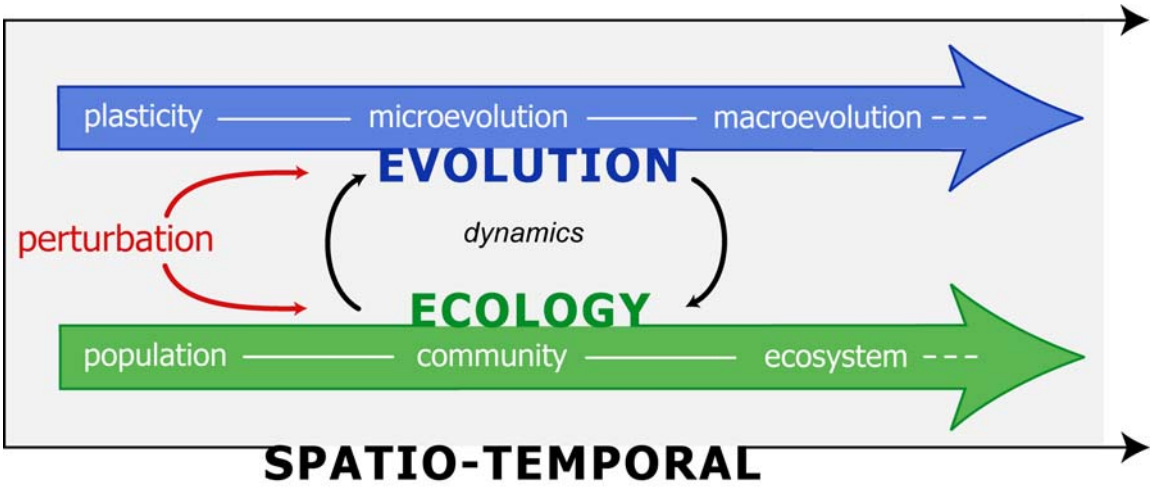

Figure 2. Schematic depicting eco-evolutionary dynamics. Blue and green arrows represent increasing scales for evolutionary (blue) and ecological (green) aspects of the dynamic, with a few representative scales written, connected by the lines indicating an increasing gradient (for example, meta-populations or meta-communities are not written, but would fall along the line in between the other levels on the ecological scale). The eco-evolutionary dynamic is depicted by the black arrows. Environmental perturbations (red arrows) can perturb either the ecological or evolutionary aspect of the dynamic. Eco-evolutionary dynamics are iterative processes that constantly occur within and across spatio-temporal scales (grey box, representing small portion of a larger spatio-temporal scale arrow).

CHAPTER 2:

THREE ROUTES TO CRYPSIS: STASIS, CONVERGENCE, AND PARALLELISM IN THE *MASTIGIAS* SPECIES COMPLEX (SCYPHOZOA, RHIZOSTOMEAE)

1 Abstract

Evolutionary inference can be complicated by morphological crypsis, particularly in open marine systems that may rapidly dissipate signals of evolutionary processes. These complications may be alleviated by studying systems with simpler histories and clearer boundaries, such as marine lakes—small bodies of seawater entirely surrounded by land. As an example, I consider the jellyfish *Mastigias* spp. which occurs in two ecotypes, one in marine lakes and one in coastal oceanic habitats, throughout the Indo-West Pacific (IWP). I tested three evolutionary hypotheses to explain the current distribution of the ecotypes: (H_1) the ecotypes originated from an ancient divergence; (H_2) the lake ecotype was derived recently from the ocean ecotype during a single divergence event; and (H_3) the lake ecotype was derived from multiple, recent, independent, divergences. I collected specimens from 21 locations throughout the IWP, reconstructed multilocus phylogenetic and intraspecific relationships, and measured variation in up to 40 morphological characters. The species tree reveals three reciprocally monophyletic regional clades, two of which contain ocean and lake ecotypes, suggesting repeated, independent evolution of coastal ancestors into marine lake ecotypes, consistent with H_3; hypothesis testing and an intraspecific haplotype network analysis of samples from Palau reaffirms this result. Phylogenetic character mapping strongly correlates morphology to environment rather than lineage ($r = 0.7512$, $p < 0.00001$). Considering also the deeper relationships among regional clades, morphological similarity in *Mastigias* spp. clearly results from three separate patterns of evolution: morphological stasis in ocean medusae, convergence of lake morphology across distinct species and parallelism between lake morphologies within species. That three evolutionary routes each result in crypsis illustrates the challenges of interpreting evolutionary processes from patterns of biogeography and diversity in the seas. Identifying cryptic species is only the first step in understanding these processes; an equally important second step is exploring and understanding the processes and patterns that create crypsis.

2 Introduction

Reconstructing ancestral phenotypes and patterns of descent is a difficult problem in evolutionary biology (Losos, 2011a). Phylogenetic inference can be complicated by extremes in morphological variance that conceal links between appearance and genetic

relationships. The difficulties caused by such phylogenetically uninformative or misleading morphological variance are acute in marine environments where cryptic species are common (Knowlton, 2000; Bickford et al., 2007). Estimates of the proportion of marine species that are cryptic (i.e., morphologically difficult to distinguish (Knowlton, 2000; Appeltans et al., 2012) and thus taxonomically classified together) range as high as 55% for some taxa (Appeltans et al., 2012), possibly a result of the prevailing physical structure of marine environments (Hamner, 1995) and the primarily non-visual mating cues used by many marine species to recognize conspecifics (Knowlton, 2000). Consequently, morphological crypsis can mask species boundaries, despite deep evolutionary divergences (Goetze, 2003; Bickford et al., 2007; Shenkar and Swalla, 2011; Singhal and Moritz, 2013).

The difficulties of evolutionary reconstruction raised by a high incidence of cryptic species have been compounded by uncertainty about the geographic distribution of variation in marine systems. A dearth of clear geographic discontinuities in the ocean led to the conclusion that marine species generally have high dispersal, relatively rare population differentiation, and an elevated proportion of ecological speciation via adaptation to local environments (Palumbi, 1994; Carr et al., 2003; Bowen et al., 2013). However, potentially important roles for limited dispersal have been emphasized (Cowen et al., 2000; Buonaccorsi et al., 2004; Cowen and Sponaugle, 2009), and there are many short-range endemics (Bird et al., 2007; Froukh and Kochzius, 2007; Wood and Gardner, 2007). These mixed results likely indicate that multiple mechanisms and patterns of evolution occur on various scales in the oceans (Palumbi, 1994; Dawson and Hamner, 2008; Bowen et al., 2013).

These challenges for evolutionary inference embodied in cryptic species may be overcome in three ways. First, molecular analyses have been used as an important, albeit also imperfect (Wheeler et al., 2013), complement to morphological data for reconstructing lineages and their histories (Losos, 1999). Second, the clearest geographic situations have been sought out for study, oftentimes on terrestrial islands and in freshwater lakes, where the evolutionary systems are clearly bounded (Baldwin et al., 1990; Meyer, 1993; Schluter and Nagel, 1995; Losos et al., 1998; Seehausen, 2006; Grant and Grant, 2009; Losos, 2009). Third, studying incipient species has provided opportunities to explore factors influencing divergence of lineages on micro-evolutionary timescales, when patterns have been least obscured by the passage of time (Lynch, 1989; Via, 2009). Molecular analyses of species inhabiting relatively young islands, therefore, may provide particularly clear situations—to the extent that islands are microcosms of more complex mainland systems (MacArthur and Wilson, 1967)—for studying the factors that influence morphological evolution.

Marine lakes—small bodies of seawater surrounded entirely by land—may provide this kind of opportunity for studying marine organisms. Marine lakes act as ‘islands’ to an oceanic ‘mainland’ (Dawson and Hamner, 2005), serving as natural laboratories inhabited by a suite of marine coastal organisms (Dawson et al., 2009). Like their freshwater lake and terrestrial island counterparts, replication of these marine islands provides a clear framework for testing specific hypotheses (Dawson et al., 2016).

Here, I explore a case study: the morphological evolution of a scyphozoan jellyfish, *Mastigias* spp. (Rhizostomeae, Mastigiidae). *Mastigias* medusae are found

throughout the Indo-West Pacific (IWP; Kramp, 1961) and occur as two ecotypes: one primarily in coastal waters including semi-protected coves and lagoons (hereforth called "ocean"), and the other in marine lakes ("lakes") (Dawson et al., 2009). Despite multiple described species of *Mastigias* in the IWP (Mayer 1910; Kramp 1961), most recent observations are ascribed to *M. papua* (e.g. Dawson, 2005b; Lewis et al., 2008; Saravanan et al., 2013; Becking et al., 2015) irrespective of ecotype or geographic location. Adding to the taxonomic uncertainty, *Mastigias* from disparate ocean locations appear predominately similar (Fig. 1a, c, e), as do *Mastigias* from disparate lake locations (Fig. 1b, d). Moreover, each *Mastigias* ecotype occurs proximate to the other ecotype throughout the IWP—for example, both are found in Palau, in Papua, and in Berau (Fig. 1; Dawson et al., 2009). Thus, in addition to taxonomic uncertainty, this pattern also raises the question: how did the morphologically most distinct medusae become the geographically most proximate (and vice versa)? Scyphozoans, like many other marine invertebrates, show high incidence of cryptic species (Dawson and Jacobs, 2001; Dawson, 2004; Holland et al., 2004; Appletans et al., 2012), but case studies for the taxon are sparse. Preliminary molecular analyses of *M. papua* in Palau suggest repeated evolution of the marine lake ecotype from an ocean ancestor since the Last Glacial Maximum (LGM; Dawson and Hamner, 2005), when modern marine lakes formed (Dawson et al., 2009; Smittenberg et al., 2011), and suggest evolution of morphologically distinct ecotypes consistent with subspecies (Dawson 2005b). It is unknown, however, if the morphological similarity within each *Mastigias* ecotype across the IWP is a plesiomorphic consequence of shared ancestry or a homoplastic result of convergent or of parallel evolution.

To examine these questions, I combine molecular phylogenetic and phylogeographic analyses of *Mastigias* spp. with descriptions of their morphological variation across the IWP and within the Palau archipelago. I test three hypotheses which could explain the morphological similarity within, and disparity between, ecotypes of *Mastigias* spp. across the IWP (Fig. 2).

H_1: The lake and ocean ecotypes are from an ancient divergence; potentially they may even be separate species of *Mastigias*. In this scenario, the two types diverged at least prior to the LGM, with the lake ecotype subsequently colonizing multiple marine lakes. Although geologic evidence suggests the current marine lakes did not exist prior to the LGM (Smittenberg et al., 2011), refugia lakes could have existed (e.g. see Grigg et al., 2002). The lake ecotype might have survived the LGM by living in refugia lakes and subsequently dispersed into newly formed, modern-day marine lakes as the refugia lakes became inundated with the rising sea-level.

H_2: The two *Mastigias* ecotypes are separated by one single, post-glacial transition between ecotypes. In this scenario, the lake ecotype arose from a recent colonization event from the ocean into one modern, post-glacial marine lake, followed by divergence into the lake ecotype and its subsequent dispersal to other lakes throughout the IWP. The long-lived pelagic medusa stage which brood planula larvae until the larvae are near ready to settle on the benthos (Uchida, 1926) provides a possible mechanism for successful long-distance dispersal and colonization.

H_3: The lake ecotype evolved by repeated and independent colonization of lakes across the IWP from adjacent coastal populations, resulting in multiple transitions

between ecotypes. As the marine lakes were formed, each one was independently colonized from the adjacent source population. As conduits into the lakes closed off or lakes became otherwise isolated, the jellyfish in each lake independently diverged into the lake ecotype. Lake ecotypes would not occur in the ocean nor disperse between lakes.

To distinguish among these hypotheses, I map morphological variation among *Mastigias* onto their molecular phylogeny. I aim to identify the best supported hypothesis of morphological evolution in *Mastigias*, and to consider whether this result may provide some insight into patterns of evolution in the seas.

3 Methods

3.1 Study region and system

Marine lakes occur throughout the world in coastal regions where karst topography is present, with a large assemblage in the Indo-West Pacific including Palau (Hamner and Hamner, 1998; Dawson et al., 2009). Sedimentation rates and ^{14}C-dated sediment cores in Palau, and lake bathymetry more broadly, indicate modern marine lakes formed after the LGM as rising sea levels flooded through cracks into low-lying areas within islands (Dawson et al., 2009; Smittenberg et al., 2011). Modern marine lakes vary in the timing and amplitude of tides relative to the ocean illustrating different degrees of connectivity (Hamner and Hamner, 1998) which, combined with varying depths, cause marine lakes to exhibit a variety of physical environments, from holomictic and ocean-like to stratified (e.g. increasing dysoxia with depth) to meromictic i.e. stratified with an anoxic layer below a sharp chemocline (Hamner and Hamner, 1998; Cerrano et al., 2006; Becking et al., 2011).

3.2 Molecular analyses: extraction, amplification, sequencing and alignment

For phylogenetic analyses, tissue was biopsied from the oral arms of 60 *Mastigias* and two of its sister taxon *Phyllorhiza punctata* (Bayha et al., 2010) (Table 1, Fig. 3). For a detailed intraspecific analysis, tissue samples were biopsied from an additional 157 medusae from 15 locations in Palau (Table 1); 15 specimens or the highest number available were used per location. All tissues were preserved in 95% ethanol and stored at -20°C.

DNA was extracted using a CTAB-phenol/chloroform protocol (Dawson and Jacobs, 2001) and used in 50 µl polymerase chain reactions (PCRs) to amplify five molecular markers: mitochondrial cytochrome *c* oxidase subunits I (COI) and III (COIII), 16S rDNA, and nuclear histone 3 (H3) and 28S rDNA; preliminary analyses indicated 28S rDNA contained only rare autapomorphies within *Mastigias* spp. and therefore was not analyzed further. COI was amplified using LCOjf (Dawson and Hamner, 2005) paired with MpHCO (Dawson and Hamner, 2005), HCO2198 (Folmer et al., 1994) or Aa_HCOI_12582 (Bayha and Dawson, 2010). 16S was amplified with 16S-L (Ender and Schierwater, 2003) and Aa_H16S_15141H (Bayha and Dawson, 2010); H3 was initially

amplified with Histone 3 F Deg and Histone 3 R Deg (Colgan et al., 1998) until specific primers H3_Mp_9F (CGACCGGAGGAAAGGCACCC) and H3_Mp_327R (CGTTTTGCGTGGATGGCGC) were designed. COIII was initially amplified with COIIIA and COIIIB (O'Foighil, unpublished) until specific primers were designed which paired COIII_Mp_6670b (ACGTAGCGTCGTGTGGAAC) with either COIII_Mp_7339Rdeg (ATAYCAACTGGCTGCYTCAAACC) or COIII_Mp_7339R (ATACCAACTGGCTGCCTCAAACC). All PCR reactions were composed of 0.5 µM primers, 5.0 µl 10x PCR buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 µl Taq polymerase (Applied Biosystems, Foster City, CA), and 1 µl sample and run on an Applied Biosystems 2720 thermal cycler. All PCRs of COIII and H3, and occasional PCRs of COI and 16S, also included 0.2 mM Bovine Serum Albumin (BSA). All PCRs included at least one initial hold of 94ºC of 3–5 min, then between 33–39 cycles that included denaturation at 94ºC for 30–45s, annealing between 50–56ºC for 30–60s, and extension at 72ºC for 60–90s, before a final hold of 72ºC for 10 min. Primer sequences and thermocycles specific for each locus and primer pair are given in Appendix I.

Amplicons of COIII, during primer development, and H3, throughout the study, were cloned using pGEM-T vector (Promega, CA) and One Shot TOP10 competent cells (Invitrogen, Life Technologies Corp.) using the pGEM-T protocol (Promega, CA) and DNA was purified using Invitrogen's PureLink MiniPrep Kit after cultures were grown in TB overnight. DNA then was sequenced, or for amplicons of all other markers, cleaned with ExoSap and sequenced, at the University of Washington's High-Throughput Genomics Unit, at the University of California, Berkeley's DNA Sequencing Facility, or at Macrogen.

Sequences were trimmed of primers, assembled into contigs, and inspected visually to check base calls and open reading frames using Sequencher v. 4.1 (Gene Codes Corporation, Michigan, USA). Sequences were confirmed to have high sequence similarity with *Mastigias* or other scyphozoans by BLASTn (Altschul et al., 1997). All loci were then aligned in MUSCLE (Edgar, 2004) using default parameters and checked by eye. 16S was aligned using three additional strategies: using MUSCLE (Edgar 2004) with six combinations of gap opening and extensions penalties ranging from -1,000 to -100 and -16 to -0.05 respectively; in MAFFT (Katoh et al., 2005) with the E-INS-i algorithm using default parameters for the offset and gap opening values set to either default, 1.0, or 3.0; and in TCOFFEE using default parameter settings (Notredame et al., 2000). All 16S alignments listed above were compared in GBLOCKS using default parameters and ambiguously aligned regions were excluded from further analyses, leaving only regions that were aligned robustly across alignments (Castresana, 2000; Talavera and Castresana, 2007). Sequences were deposited in GenBank (KU900912–KU901464).

3.3 Molecular analyses: phylogenetic reconstruction and intraspecific networks

Initial gene tree analyses showed locus H3 sequences form two distinct clusters and BLASTn results suggested two paralogous H3 loci, which we denoted as H3a and H3b and analyzed separately. Trees were reconstructed with *Phyllorhiza*—the sister taxon to *Mastigias*—as the outgroup to polarize character evolution, and also without *Phyllorhiza* in case of effects of long-branch attraction, per Bergsten (2005). The best fitting model of

sequence evolution for each of the mtDNA and nDNA markers (HKY+I for all loci except HKY+Γ for H3a and HKY for H3b) was selected using corrected Akaike Information Criterion and Bayesian Information Criterion delta scores in jMODELTEST (Posada, 2008). Maximum likelihood gene trees for each locus were constructed and bootstrapped 1,000 times on the CIPRES portal (Miller et al., 2010) using GARLI 2.0 (Zwickl, 2006). A majority-rule consensus of bootstrap trees from GARLI was compiled in PAUP* v. 4.0b10 (Swofford, 2002). Population expansion in each mitochondrial clade was tested using Extended Bayesian Skyline Plots prior to Bayesian gene tree construction in BEAST v. 1.7.5 (Drummond and Rambaut, 2007), which used priors from jMODELTEST. Bayesian trees were constructed with all assumptions of clocks and population growth combinations, and then examined in Tracer v. 1.5 (Rambaut and Drummond, 2007) to determine that the assumptions of a relaxed clock with a logistically growing population returned the most resolved tree. Bayesian Markov Chain Monte Carlo methods were run for 100 million iterations, with samples taken every 1,000 steps and the first 10,000 trees discarded as burn-in. All Bayesian chains were run at least twice; there was no difference in topology or supported branches' lengths between runs. Sample trees were re-sampled using LogCombiner in the BEAST package, excluding burn-in, to produce a final amount of 10,000 trees. Convergence was assessed using Tracer v. 1.5 (Rambaut and Drummond, 2007).

Species trees were reconstructed using Bayesian and maximum likelihood analyses of two datasets: (1) partitioned matrices, with models of evolution specified for each locus, and (2) from unlinked, individual gene trees. Partitioned trees, for (1), were generated (a) using maximum likelihood on CIPRES' GARLI 2.0 (Zwickl, 2006; Miller et al., 2010) with a majority-rule consensus of 1,000 bootstrap trees compiled in PAUP* (v.4.0b10 Swofford, 2002) and (b) using Bayesian analyses in BEAST v.1.7.5 (Drummond and Rambaut, 2007). Two species trees, for (2), were constructed using Bayesian analysis in *BEAST (Drummond and Rambaut, 2007) for (a) a dataset of only loci with all specimens and (b) a dataset of only specimens with data for all loci. All Bayesian analyses were each run twice, for 100 million iterations with samples taken every 1,000 iterations. The resulting trees were thinned in LogCombiner after removing a 10,000 iteration burn-in to produce the final 10,000 trees. Convergence was tested before and after the thinning using Tracer v.1.5; there was no difference between runs in the final tree's topology or supported branches' lengths.

Agreement in tree topology between reconstruction methods within loci and between loci within methods was assessed using COMPARE2TREES v. Sept 2011 (Nye et al., 2006) using trees with only supported nodes with ≥70 posterior probability or bootstrap support. Overall topological similarity was measured by calculating a percentage of matched branches between two compared trees (Nye et al., 2006).

Detailed intraspecific analyses were performed on an expanded Palau dataset since it could provide greater resolution between locations within a species and region, due to larger sample size of many ocean and lake locations, than the phylogenetic analyses. Intraspecific relationships within Palau were represented using statistical parsimony analyses of COI and COIII loci independently, as well as concatenated, in TCS (Clement et al., 2000). Population differentiation was estimated by calculating Φ_{ST} between locations using Arlequin v. 3.5.1.2 (Excoffier and Schnieder, 2005).

3.4 Morphological analyses

3.4.1 Specimen collection

The morphological dataset included a total of 262 medusae. Existing data from 183 *Mastigias* from Palau (Dawson, 2005a) were combined with new data from seventy-nine *Mastigias* medusae ranging between 20–160 mm bell diameter (per Dawson, 2005a) collected from a total of 16 locations across the IWP (Table 1, Fig. 3). Whole animals were fixed in 4% formalin with seawater. Additionally, 116 *Mastigias* (Table 1, Fig. 3), including some at locations from which preserved medusae were unavailable, were measured from photographs taken of medusae *in situ* with the oral-aboral axis perpendicular to the photographer. Overall size was measured *in situ* on 33 medusae from ocean locations in Palau for comparison to existing population size data from lake locations. Comparable data for the sister taxon *Phyllorhiza* were collected from one specimen and five photographs.

3.4.2 Morphological measurements

Morphological features described by Dawson (2005a), except characters on coloration and mass (*f1* to *f8*), were measured on preserved specimens. Photographed individuals allowed measurement of only six externally visible continuous morphological features (*f9-f12, f14-f15* from Dawson, 2005a) using JMicroVision v. 1.2.7 (Roduit), but did enable scoring of additional coloration and meristic features (*f1-f7*; Dawson, 2005a). One character, mean terminal club length, was correlated with the terminal club number; a complete absence of terminal clubs would result in "missing data" for length, thus creating a bias for any analyses which would exclude any specimens with missing data. To correct for this, I calculated a new metric by multiplying terminal club number by mean terminal club length. Since photographs had different resolutions and magnifications, I standardized all quantitative measures that are isometric with size (Dawson, 2005a) as ratios of the bell diameter. Each character was tested for normality and homoscedasticity in R v. 2.11.1 (R Development Core Team, 2010) or, for small samples ($n < 5$), the median and mean were compared and small differences taken as a rough approximation of normality. All samples met the assumptions of normality and homogeneity of variance.

3.4.3 Construction of data matrices

The fragility of jellyfish makes preservation difficult, and many specimens had some damage which necessitated coding one or more traits as missing data. To maximize inclusion of specimens in non-metric Multidimensional Scaling (nMDS) analyses, which do not permit missing data, I reduced the number of traits in the matrix. To do so objectively, I initially performed a Principal Component Analysis (PCA) on those individuals without any missing data to determine which of the 35 non-color related

characters contained the most informative, non-redundant data (R v. 2.11.1, prcomp(), stats package, R Development Core Team, 2010). I then grouped characters within 5-degree arcs around the PCA origin into clusters. Any character which fell in more than one cluster was assigned to the cluster to which it was closest. Finally, the character with the strongest signal in each cluster was considered relative to other clusters; only those that were >40% of the strongest overall—which was a natural gap between strong and weak signals—were included in the final matrix for nMDS analyses. To assess the impact of this data reduction step on subsequent inferences, I conducted sensitivity analyses by repeating the procedure using 2.5-degree or 10-degree arcs and using 30% or 50% cutoffs for signal strength. In all exploratory tests, these sensitivity analyses showed that samples occupied the same relative positions on nMDS plots and that the final matrix (5-degree, 40% cutoff) was statistically indistinguishable from those using more characters and alternative groupings. I performed this data-reduction on a dataset containing specimens from across the IWP and on another containing specimens from only Palau, generating two matrices for nMDS analyses: (1) *IWP1* which has 13 independently informative characters for 203 individuals across the IWP, and (2) *PW1* which has 15 independently informative characters for 136 specimens in Palau. Subsequently, I also performed the character-reduction including photographed specimens with only external characters, and thus representing every location, resulting in two additional matrices: (3) *IWP2* which had five independently informative characters for 250 IWP specimens, and (4) *PW2* which had five characters for 169 Palau specimens.

3.4.4 nMDS analyses

nMDS analyses were performed in Primer v. 5.2.9 (Clarke and Gorley, 2001) using Bray-Curtis similarities. Each nMDS analysis used ten randomly seeded starts to assess repeatability of the results (Clarke, 1993) and minimum stress results used to assess the data representation quality. Agreement between analyses of the paired datasets (*IWP1* and *IWP2*, *PW1* and *PW2*) was assessed by the similarity in clustering, as well as PERMANOVA calculations of nested environment and location variables (R v. 2.11.1, adonis(), vegan package; Oksanen et al., 2011). Palau had the most complete sampling coverage from multiple cove and lake locations, so I additionally performed post-hoc Tukey tests on the nMDS distributions along each axis from *PW2* to determine the specific locations driving the overall pattern (R v. 2.11.1, TukeyHSD(), stats package, R Development Core Team, 2010). P-values within each analysis set were considered after Sequential Bonferroni correction (Rice, 1989).

An estimate of overall phenotypic similarity among samples across the IWP was calculated as the distance on the *IWP2* nMDS plot of each datapoint from the minimum X and Y values on the plot (i.e. sqrt $((x_p - x_{min}) + (y_p - y_{min}))$). Thus, points with a similar distance were closer together on the nMDS and therefore phenotypically similar; boxplots show the variation in overall similarity within a site.

3.5 Hypothesis testing, character state reconstruction and phylogenetic contrasts

To test consistency of the species tree with each of the three alternative hypotheses, I compared the overall topological similarity of the species tree with each of the representative hypothesis trees drawn in Fig. 2. Since the hypothesis trees tips are identified only by ecotype and region, I reclassified specimens in the species tree similarly. Then, within each region and ecotype pair, I randomly assigned on each tree an additional identifier per individual to create a unique name (e.g. Region1-Lake-F, Region2-Ocean-D). Branch lengths were generated for each hypothesis tree by scaling the tree to the species tree's molecular clock based on the positioning of the hypothesized divergence relative to the Last Glacial Maximum. Each hypothesis tree was compared to the species tree using COMPARE2TREES v. Sept 2011 (Nye et al., 2006) to generate an overall topological similarity score.

I inferred ancestral morphological states across the species tree using squared-change parsimony reconstruction in Mesquite v. 2.75 (Maddison and Maddison, 2011). Since only the genetic samples from Raja Ampat and Japan had corresponding samples in the morphological dataset, the character mean value for each location was used. Categorical values were converted to 0 or 1 binomials or hierarchical number classes to calculate a mean. Overall phenotypic similarity was calculated as the mean values of the linearized distance on nMDS analyses for each location. Those overall mean values, as well as mean values of each categorical and continuous character individually (regardless of coverage), were mapped across the species tree. I estimated correlation between phenotypic similarity and environment type (ocean/lake) using Felsenstein's Independent Contrasts executed in the PDAP package of Mesquite (Midford et al., 2010) after reducing the degrees of freedom due to uncertainty at nodes with low support (Purvis and Garland, 1993). P-values across these ancestral state reconstructions were considered after Sequential Bonferroni correction (Rice, 1989).

4 Results

4.1 Phylogenetic analyses

All gene tree reconstructions except that for locus H3, which had low overall resolution (Appendix II), and all species trees grouped *Mastigias* specimens into three, strongly supported, deeply divergent clades: one "China Seas clade," including Japan, Berau and Vietnam; a second "Pacific Islands clade," including Palau, West Papua and Enewetak; and a third "Solomon Sea clade," exclusive to Tufi (Fig. 4; Appendix II). The general topology of gene trees was consistent between methods within loci (overall topology similarities = 100%) and between loci (overall topology similarities ≥ 70%). The same topology was produced in the Maximum Likelihood and Bayesian partitioned dataset species trees and with the Bayesian species tree (overall topology similarities ≥ 98%; Fig. 4). The consensus topology for each gene tree and the species tree suggests that the Solomon Sea clade branches basally relative to the other two main clades and

that there is considerable divergence between all three clades (Fig. 4; species tree with *Phyllorhiza* outgroup, Appendix III).

There was greater divergence between clades than between ecotypes. The between clade mean estimated divergence (mean substitutions per site) of the partitioned dataset was 0.071 and 0.067 between Solomon Sea clade and the China Seas clade and the Pacific Islands clade, respectively. The divergence between the China Seas and Pacific Islands clades was 0.049. The within-clade mean distance, which includes the difference from ocean and lake ecotypes, is decidedly less, with the Solomon Sea clade at 0.005, the China Seas clade at 0.010, and the Pacific Islands clade at 0.006. Between clade mean estimated divergence in COI and COIII datasets ranged between 0.071–0.124. With the exclusion of the Solomon Seas clade, which was comprised of only two Tufi samples, the remaining clades included both lake and cove locations within a given region.

Within Palau, both COI and COIII haplotype networks show central and diverse ocean haplotypes with lake haplotypes on the periphery of the network (Appendix IV), as does the concatenated mitochondrial network (Fig. 5). Lake locations typically have only one to three unique haplotypes each, whereas cove locations are more diverse and share common haplotypes across locations (Fig. 5); the exception to this pattern is Ongeim'l Tketau (OTM) which has multiple rare haplotypes. Calculation of Φ_{ST} between locations reveals that ocean sites, Mekeald Lake (MLN) and Tketau Lake (TLM) have zero or weak genetic differentiation (Appendix V). In contrast, comparisons between all ocean locations with each meromictic lake and with T-Lake (TLN) and Ongael Lake (OLO), as well as TLN, OLO and meromictic lakes with each other, have $\Phi_{ST} > 0.2$ and $p \leq 0.0028$.

4.2 Morphological analyses

The nMDS analyses of the *IWP1* and *IWP2* grouped specimens by environment (ocean vs. lake) regardless of region (Fig. 6a, Appendix VI), with stress scores indicating the plot represented the respective data matrix well (*IWP1* stress = 0.07; *IWP2* stress = 0.02). Additionally, PERMANOVA calculations show a significant ($p < 0.001$) clustering by environment in each matrix. For *IWP1*, environment (ocean vs. lake) explained 15% of the total variance, and locations within environments another 51%; for *IWP2,* the variance explained by environment is 47% and locations within environments 33%. Estimates of overall phenotypic similarity display the two ecotypes distinct from each other, although with a degree of variation within ecotypes (Fig. 6b).

Generally, medusae with the ocean ecotype display a high degree of pigmentation in the bell, oral arms and terminal clubs, including an abundance of colored spots on the bell and flecks in the canals. Medusae with the ocean ecotype are typically large (median bell diameter 187.5 mm, maximum bell diameter 230 mm in Palau ocean locations) and generally have all of the 8 terminal clubs (median = 8, mean = 7.5) which are just over half the length of the bell diameter. They are also fairly robust, with the oral pillars, oral disc and bells that are between 15 and 30% thicker and a canal system that has 2.5–5 times more anastomoses than the lake ecotype medusae. In addition, medusae with the lake ecotype are typically smaller (Dawson, 2005b) and have lost most or all of the pigmentation found in the ocean medusae. They have a reduction in the number, length

and thickness of the terminal clubs, with a median 7 of 8 terminal clubs (mean = 6.3), although older lakes often have medusae with no terminal clubs, and when present, the terminal clubs are only about 0.17 of the bell diameter in length.

The nMDS analyses of all Palau medusae show meromictic lake locations cluster together, as do holomictic and 'stratified' lakes (stratified, but not meromictic) with ocean locations (*PW1* stress = 0.08; *PW2* stress = 0.02; Appendix VI). The PERMANOVA tests indicate that specimens within each nMDS clustered significantly by environment ($p < 0.001$). *PW1* had 14% of the variance explained by environment (ocean versus lake) and 50% explained by locations within environments; *PW2* had 51% of the variation explained by environment and 30% by locations within environments. Post-hoc tests of the *PW2* nMDS analysis indicated that the majority of the difference between locations was on Dimension 1 (although variance on Dimension 2 is greater in lakes, the means are statistically indistinguishable for all but one pair of meromictic lakes; Appendix VII). On Dimension 1, pair-wise comparisons were significantly different ($p < 0.0003$) in 15 of 16 paired comparisons between ocean sites and meromictic lakes, 4/4 of comparisons between holomictic and meromictic lakes, 3/4 of the comparisons between 'stratified' and meromictic lakes and 4/6 of comparisons between different meromictic lakes; comparisons between ocean sites (n = 6 pairs), between ocean sites and holomictic and 'stratified' lakes (n = 8) and between holomictic and 'stratified' lakes (n = 1) were never significant ($p > 0.001$) (Appendix VII).

4.3 Hypothesis testing, character state reconstruction, and phylogenetic contrasts

The overall topological similarity of the species tree to H_1 was 36.4%, to H_2 was 35% and to H_3 was 82.7%. The scores are not a probability of the veracity of the hypothesis, but rather indicative of its value only relative to the other hypotheses; H_3 was more than twice as good as either other hypothesis. Character state reconstruction indicates that both lake and ocean ecotypes are found in each well-sampled clade of the tree (i.e., excluding the Solomon Sea clade, which has only two individuals). The lake ecotype (dark shades; Fig. 6c) is found multiple times across the tips of the phylogenetic tree; the ocean ecotype (light shades) is found throughout the tree, from tips through to deeper nodes. The ancestral morphological state is reconstructed unequivocally as an ocean ecotype. Not only is the ocean ecotype reconstructed as the ancestral morphological state for the entire tree, but also within each of the principal clades. The lake ecotype only occurs at the tips of the tree and the character state reconstruction switches to an ocean ecotype usually (6/7 times) within two nodes back on the tree.

Phylogenetic contrasts indicate a significant correlation between overall mean phenotypic similarity (Fig. 6b) and environment ($r = 0.7512$, $p = 6.514 \times 10^{-9}$; Fig. 6c). Morphology and environment were significantly correlated in 19 of 48 individual characters ($-0.4 \geq r \geq 0.4$ and $p < 0.0013$; Appendix VIII). Nine of 16 categorical morphological characters significantly correlated with environment; significant characters included those related to pigmentation. Ten (of a total possible 32) continuous characters were significant, including terminal club length, metrics regarding internal canal complexity and oral pillar dimensions. Oral arm length was marginally significant across all regions ($p = 0.017$, but not within the Sequential Bonferroni correction).

5 Discussion

5.1 Hypothesis evaluation

Mastigias medusae occur as morphologically distinct 'ocean' and 'lake' ecotypes in geographically proximate locations across the Indo-West Pacific. *Mastigias* in the IWP also form a complex of at least three regionally distinct and reciprocally monophyletic clades, which are separated by genetic distances equivalent to species-level differences in some other jellyfishes (Bayha and Dawson, 2010; Ortman et al., 2010). Thus, the two ecotypes are not restricted to distinct clades and are not distinguished by a single ancient nor a single recent divergence, refuting my hypotheses H_1 and H_2.

Rather, both ecotypes occur within multiple species-level lineages; each well-sampled species contains lake and ocean ecotypes, with each lake population most closely related genetically to its nearby ocean population (Fig. 4). The transitions between ocean and lake ecotypes occur near the tips of the species tree, indicating multiple recent transitions (Fig. 6c). This interpretation is supported by detailed evidence from Palau that shows lake ecotypes are recently derived from ocean ecotypes, have low genetic diversity, and low gene flow (Fig. 5), and supported by the species tree having greatest similarity with the H_3 hypothesis tree. The multiple transitions between closely-related ocean and lake populations is strong evidence supporting H_3: recent, repeated, independent evolution from coastal waters into marine lakes. The broader phylogenetic analysis that shows at least two reciprocally monophyletic lineages of *Mastigias* across the IWP, both with ocean and lake ecotypes, indicates modern *Mastigias* became morphologically cryptic via three routes: stasis, convergence and parallelism.

5.2 Three routes to crypsis

The high incidence of cryptic species in marine environments may confound attempts to infer patterns of descent from morphology and thus inhibit understanding of evolutionary patterns and processes in the seas (Bickford et al., 2007; Knowlton, 2000). In *Mastigias* spp., morphological similarity within habitat types could be a plesiomorphic consequence of shared ancestry, attributable to synapomorphies, or a homoplastic result of convergent or of parallel evolution. Ancestral state reconstruction reveals that crypsis in *Mastigias* is a result of both retention of ancestral characteristics and *de novo* evolution of homoplasies. While these, like all ancestral character reconstructions, are hypotheses, they are robust in that characters are mapped across strongly supported nodes in the species tree and most individual characters show the same pattern as the overall morphology (Fig. 6c). Plesiomorphic features and stasis characterize ocean ecotypes along species lineages; lake ecotypes are characterized by homoplasious *de novo* evolution, either through convergent evolution between species or through parallel evolution within species.

Stasis, parallelism, and convergence are relative terms. In the context of this study, they describe the broad patterns present in *Mastigias* and which could be present in other marine organisms. While there is discussion about how, when, and at what scale the

terms apply (e.g. Arendt and Reznick, 2008), I find that distinguishing between the terms is useful when setting up different evolutionary questions as long as the scale is specified (Elmer and Meyer, 2011). In this sense, I use the terms to distinguish patterns of morphological evolution relative to the underlying phylogeny. Stasis is used to mean the retention of an ancestral phenotype despite the accumulation of genetic differentiation; parallelism is repeated independent evolution of a phenotype within a lineage; convergence is independent evolution of a phenotype in different lineages. Critically, parallelism evinces repeated evolution of a trait state from one recent common ancestor, whereas convergence requires traits evolve after divergence from the most recent common ancestor and establishment of the lineages being compared. That 'lineage' can apply at various scales on the tree of life is consistent with the idea that all scales of comparisons are valid (Elmer and Meyer, 2011; Lescak et al., 2015; Westram et al., 2014) and thus I apply stasis, parallelism, and convergence at the intrageneric scale to patterns of morphological evolution along lineages within *Mastigias* as an example of the kinds of evolution one can see in marine taxa.

5.2.1 Crypsis through stasis

Morphological stasis in *Mastigias* is displayed in the similarity of ocean species across the IWP. The morphology of ocean ecotypes was statistically indistinguishable across clades and grouped tightly on the *IWP1* and *IWP2* nMDS analyses. Critically, phylogenetic character reconstruction indicates that the common ancestor of the three *Mastigias* species lineages had an ocean ecotype. Assuming approximately 0.65% sequence evolution per million years for medusozoan COI (e.g. Govindarajan et al., 2005), which is within the general range for COI across taxa (Lessios, 2008)), the genetic differences between the three major clades of *Mastigias* indicate relative morphological stasis over ~6 million years of independent evolution.

Morphological similarity of ocean ecotypes relates to several obvious characters that are statistically distinct from the lake ecotype and that may relate to the environmental conditions in the ocean. Ocean ecotypes are colored, most obviously with blue pigment in the bell, which also is adorned with abundant yellow or white spots; these colors also may appear in the oral arms, terminal clubs and internal canals (see Fig. 1). Similar blue pigmentation in other rhizostome jellyfish has been hypothesized to be photoprotective for symbiotic *Symbiodinium* (Blanquet and Phelan, 1987), a function also tentatively attributed to *Mastigias* (Dawson and Hamner, 2003). Photoprotective pigment may benefit the ocean medusa-zooxanthellae holobiont that daily inhabits the high-light near-surface waters, a situation that contrasts with the lake environment and medusae (Dawson and Hamner, 2003; see next paragraph). Additionally, ocean medusae are generally large and have eight long thick terminal clubs; the clubs are large relative to medusa size and considerably exceed the size of clubs of the lake ecotype. The ocean ecotype's large size and long terminal clubs, which may aid in swimming quickly and efficiently in straight lines (Dawson and Hamner, 2003; Dawson, 2005a), may benefit ocean medusae which have to maintain cohesive populations in the face of a tendency to be dispersed or advected away by tidal currents. Similar environmental conditions, e.g. high water clarity, advective currents, and visual predators, occur in all ocean locations

across regions and thus may create conditions for morphological stasis despite genetic divergence.

5.2.2 Crypsis through convergence

The lake ecotypes are morphologically similar across the IWP (Fig. 6a) despite a deep evolutionary divide between lake ecotypes in different species (e.g. Palau vs. Berau) and despite the most recent common ancestor of each species being an ocean ecotype (Fig. 6c). Thus, morphological similarity of the lake ecotype across the IWP, at least between species, arose via convergence.

Within meromictic lakes across regions, the suite of similar morphological characters is the converse of the characteristic ocean ecotype: lake ecotypes *generally* exhibit a reduction in pigmentation, in the number, length and thickness of terminal clubs, and in overall size (see: Dawson, 2005a; Fig. 1), although, there is greater variation in phenotype in the lakes than in the ocean ecotype (Fig. 6a) due to both greater variation within populations (Fig. 6a, b) and the larger number of isolated populations in the lake ecotype (Fig. 5, Fig. 6c). The reduction or loss of pigmentation in Palau lake medusae has been linked with the evolution of behavioral photoprotection in higher turbidity lakes (Dawson and Hamner, 2003), which was hypothesized to possibly allow finer and more immediate control of sunlight exposure, although the exact sequence of evolution of the two is unclear (Dawson and Hamner, 2003). The reduction in size and in terminal clubs, combined with a broader, slightly flattened bell shape, should increase drag from a less streamlined body that could explain reduced efficiency and speed of swimming in the lake ecotype (see Dawson and Hamner, 2005), and may be related to the lack of a selective force to maintain cohesive populations in lake environments (Dawson, 2005a; Dawson and Hamner, 2003;). Oral arm length is marginally significantly correlated with environment across regions and highly significant within Palau, indicating an effect of environment, at least when areas are studied in detail, or an effect of non-random gene flow into a novel environment (Edelaar and Bolnick, 2012).

While the phylogeographic patterns are most detailed for Palau, similar processes seem to be shaping *Mastigias* in other regions. Deep meromictic lakes in Berau and in Palau show morphotypes that are far diverged from the adjacent ocean forms (see Fig. 1). Relatively shallow holomictic lakes in Raja Ampat and Palau (and for available qualitative measurements, Vietnam) show medusae morphology and genetics that are less diverged from the adjacent ocean (see also: Dawson, 2005a; Dawson and Hamner, 2005). Deeper meromictic lakes also occur in Raja Ampat and harbor medusae that also exhibit the more diverged lake morphology (Becking et al., 2015), but samples were not available for this study.

5.2.3 Crypsis through parallelism

While the similar morphology of lake ecotypes *across* species demonstrates convergent evolution, *within* species it evinces parallelism. Within both the Pacific Islands and the China Sea clades, lake populations do not group together in a single

monotypic clade, but have different independent origins, predominantly from an ancestral ocean population. For example, in the China Sea clade, the population in Hang Du Lake I in Vietnam is derived from an ocean ancestor independent of the divergence of medusae in Berau lakes from ocean ancestors; the same is true in Palau, in which lake populations cluster together morphologically on the *PW1* and *PW2* nMDS plots, but genetically show signatures of independent colonizations from the ocean (Fig. 5) and low gene flow. In Palau, the morphological evolution and distinct genetics of the more isolated lakes which do not exhibit any secondary contact with the ocean has been interpreted as incipient speciation (Dawson, 2005b). Thus, the lake ecotype morphology has arisen in parallel in each lake within *Mastigias* species.

The same kinds of morphological changes — loss of pigmentation, reduction in number, length and thickness of terminal clubs, reduction in oral arm length and overall medusa size — occur in populations in older and isolated lakes, particularly meromictic lakes (Dawson, 2005a). As noted in section 4.2.2, these similarities may be attributable to behavioral adaptation in photoprotection and landlocked habitat. In addition, oral arm length is significantly smaller in lakes than in the ocean within Palau, which may also pertain to swimming efficiency in landlocked environments (see section 4.2.2), or may be related to decreased reliance on *Symbiodinium* to meet metabolic needs (McCloskey et al., 1994), as the oral arms are the tissue that has a large concentration of the symbiotic *Symbiodinium.*

However, lake populations are not monotypic but show morphological variations on the ecotype. Even though morphology is similar within ecotype based on the nMDS, there is still a significant amount of variation between locations within ecotype. Previous studies with aquarium-raised medusae in Palau indicate that, while potentially slightly plastic with environmental conditions, most of the location-specific morphological differences are heritable and not primarily eco-phenotypic (Dawson, 2000; Dawson, 2005a). Most holomictic lake medusae have morphotypes similar to the ocean ecotype, while meromictic lake populations vary in morphology (Fig. 6a, b, Dawson, 2005a), and vary in other aspects such as behavior (Dawson and Hamner, 2003; Hamner and Hauri, 1981). Indeed, the different ages of lakes and the degrees of differentiation of independently evolving lake medusa populations suggest a progressive reduction in features from ocean to lake ecotype (Fig. 6b; Dawson and Hamner, 2005). Early stages in evolution may be indicated by the holomictic lake population in OLO, which is intermediate between the clusters of ocean and of meromictic lake medusae in the nMDS analyses (Fig. 6a, b). OLO and also TLN exhibit significant amounts of isolation, similar to meromictic lakes. That said, whether a lake is holomictic, stratified but not meromictic, or meromictic may not be as important in determining morphological evolution as the degree and duration of isolation or biotic factors; morphologies in holomictic lakes range across the nMDS dimensions (e.g. in Fig. 6a, DCG in Raja Ampat cluster near oceanic locations; OLO in Palau is transitional and DKK in Berau clusters with meromictic lakes in Palau and Berau). Depending on the matrix used (and the features included within each), the exact amount of variance ascribed to between-environments or within-environment-between-locations changes, indicating that different features respond in different ways or at different rates to the various environments.

5.3 Implications for understanding crypsis in the seas

Cryptic species are common in the ocean (Appeltans et al., 2012; Bickford et al., 2007; Knowlton, 2000). However, many are "pseudo-sibling species" that can be distinguished using biochemical, genetic or robust quantitative morphological analyses (Knowlton, 1993). Application of these tools has resulted in an increase in distinct, but morphologically similar, species identified in many taxa, including scyphozoans (Bickford et al., 2007; Dawson, 2005a; Dawson and Hamner, 2005; Knowlton, 1993, 2000; Xavier et al., 2010, this study). Using these approaches could increase the number of known species by as much as 55% in some marine taxa and 4.0–15.5% overall (Appeltans et al., 2012). Cryptic species distinction is a key step in better understanding the magnitude and distribution of marine biodiversity (Bickford et al., 2007).

Yet, distinguishing cryptic species, by itself, may not dramatically increase our understanding of evolutionary processes, biogeographic patterns, or patterns of adaptation in the seas (Bickford et al., 2007; Knowlton, 2000). The majority of publications distinguish cryptic species but not the mechanism or process causing crypsis in distinct species (Lee and Frost, 2002; Witt et al., 2003). This failure to identify the causes of crypsis is understandable given the complexity of these mechanisms in marine environments; indeed, my results for *Mastigias* suggest that too often we do not know whether crypsis is due to stasis, convergence, or parallelism. Yet, understanding both 1) whether phenotypes are the result of external or internal constraints or the result of convergent or parallel adaptations and 2) the mechanisms causing adaptation has important implications.

Understanding the patterns and processes generating crypsis should be the essential second step following distinction of cryptic species. The patterns and mechanisms which drive adaptation are fundamental to understanding when, where and how diversity arises, persists or is extirpated (Sanford and Kelly, 2011). Thus, the first step of identifying cryptic species remains important for elucidating biodiversity and identifying the evolutionarily significant units that may be affected by changing environments. However, the second step is equally important for understanding the mechanisms that generated extant biodiversity and how evolutionarily significant units may respond to future changes.

Crypsis through stasis, convergence, or parallelism could be caused by many processes involved in shaping dispersal and habitat partitioning (Bowen et al., 2013; Palumbi, 1994), by genetic or morphological constraints (Erwin, 2007; Losos, 2011b), or by the similarity in environment (Conover et al., 2009; Edwards et al., 2012; Eldredge et al., 2005; Hamner, 1995; Losos, 2011b). All three routes to crypsis—stasis, convergence, parallelism—are illustrated in the morphological evolution of *Mastigias.* Their relative frequencies in other marine taxa are unknown. Each may be commonplace, given the prevalence of crypsis (Hamner, 1995; Lee and Frost, 2002; Norris and Hull, 2012). Common selective and/or constraining forces could limit changes in morphology (Bickford et al., 2007) and create crypsis through stasis in marine organisms that experience strong selection from similar environments, as hypothesized by Hamner (1995) and shown in Lee and Frost (2002). Selective forces from similar and physically constraining environments also were hypothesized to produce a prevalence of

convergence in ocean systems (Hamner, 1995). Although marine examples of parallelism and convergence are lacking, the few existing studies have challenged assumptions about the time and geographic scales at which evolution occurs in the seas (e.g. Dawson and Hamner, 2005; Ravinet et al., 2016). Increasing recognition of the possibility of island and island-like marine environments and widespread phylogeographic structure in marine systems (Dawson, 2015; Gillespie and Clague, 2009; Hachich et al., 2015; Vermeij, 2004) should increase the awareness of researchers of opportunities for independent evolution—convergent, parallel, or divergent.

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7 Tables & Figures

Table 1. The region, location, sites, and sample sizes for analyses using photographs, whole specimens, and tissues for phylogenetic analyses. Italicized portion of acronyms are used as site identifiers for analyses of only Palau specimens. For COI and COIII, total numbers are given for the phylogenetic analyses before the slash and after the slash for phylogeographic analyses. Habitat type denotes either an ocean location or a lake; lakes are further differentiated into broad categories of either holomictic (mixed), 'stratified' (stratified with gradually decreasing hypoxia, but not meromictic) or meromictic (stratified with anoxic conditions below a chemocline).

Species	Region	Locality	Site	Abbreviation	Habitat	Lake Type	Morphology		Loci				
							Specimen	Photograph	COI	COIII	16S	H3a	H3b
Mastigias spp.	Papua	Tufi		IDORTUF	Ocean			1	2	2	2	2	2
		Raja Ampat	*Unknown*		Ocean			3					
			Mastigias Papua Cove	IDWPMPC	Ocean		4		5	5	5	3	5
			Gam Bay	IDWPGAM	Ocean		2		5	5	5	2	5
			DAG Lagoon Reef Flat	IDWPDGL	Ocean		1		1	1	1		1
			Danau Lake a Gam	IDWPDAG	Lake	Holomictic	1		2	2	2	1	2
			Danau Ctenophore Gam	IDWPDCG	Lake	Holomictic	1		1	1	1		1
			Rewo Bay		Ocean			1					
	Indonesia	Komodo		IDNTKOM	Ocean			2	1	1	1		1
		Berau	Haji Buang	IDBEHBM	Lake	Meromictic	15	14	2	2	2	2	2
			Tanah Bamban	IDBETBM	Lake	Holomictic	19	4	1	1	1	1	1
			Kakaban	IDBEDKK	Lake	Holomictic	12	8	2	2	2		
	Philippines	*Unknown*			Ocean			5					
	China	Hong Kong			Ocean			2					
	Micronesia	Chuuk			Ocean			1					
		Marshall Islands	Bikini Atoll		Ocean			2					
			Enewetak	MHRHEWE	Ocean			2	1	1	1	1	1
		Palau	Ngermeuangel Cove	PWKO*NCK*	Ocean		8	2	5 / 15	5 / 15	5		5
			Ngerchaol Cove	PWKO*NCN*	Ocean		12	9	1 / 15	1 / 15	1		1
			Risong Cove	PWKO*RCA*	Ocean		20	1	5 / 15	5 / 15	5		5
			Ongael Lake	PWKO*OLO*	Lake	Holomictic	27	13	4 / 15	4 / 15	4	1	3
			Goby Lake	PWKO*GLK*	Lake	Meromictic	30	4	4 / 15	4 / 15	4		4
			Clear Lake	PWKO*CLM*	Lake	Meromictic	30	7	4 / 15	4 / 15	3		
			Ongeim'l Tketau	PWKO*OTM*	Lake	Meromictic	46[b]	9	5 / 15	5 / 15	5	1	5
			Ngermeuangel Lake	PWKO*NLK*	Lake	Meromictic	20	3	6 / 15	6 / 15	6		6

Species	Region	Locality	Site	Abbreviation	Habitat	Lake Type	Morphology		Loci				
							Specimen	Photograph	COI	COIII	16S	H3a	H3b
Continued…													
			CRRF Dock	PWKO*CRF*	Ocean				0 / 9	0 / 9			
			Little Risong Cove	PWKO*LCU*	Ocean				0 / 13	0 / 13			
			Tab Ketau Cove	PWKO*TKC*	Ocean				0 / 7	0 / 7			
			Mekeald Lake	PWKO*MLN*	Lake	Holomictic			0 / 14	0 / 14			
			Tketau Lake	PWKO*TLM*	Lake	Stratified	3	1	0 / 12	0 / 12			
			T-Lake	PWKO*TLN*	Lake	Stratified		4	0 / 14	0 / 14			
			Lagoon	PWKO*LGN*	Ocean		3		0 / 2	0 / 2			
	Vietnam	Hang Du I		VNHDHDL	Lake	Holomictic[a]		2	1	1	1	1	1
	Japan	Sukumo Bay		JPKISKM	Ocean		8	12	2	2	2	2	2
		Shirahama			Ocean			1					
		Unknown			Ocean			3					
Phyllorhiza	Australia	*Unknown*			Ocean			4	2	2	2	2	2
	Borneo	*Unknown*			Ocean			1					
	Mexico	Gulf of California	La Paz	MXBSBAP	Ocean		1						

[a] Hang Du I, Vietnam has a variable rainwater surface layer associated with monsoonal rains, but is otherwise is iso-oxic with depth and thus classified as holomictic (Cerrano et al., 2006).
[b] 30 specimens from Dawson (2005a) were combined with 16 new specimens.

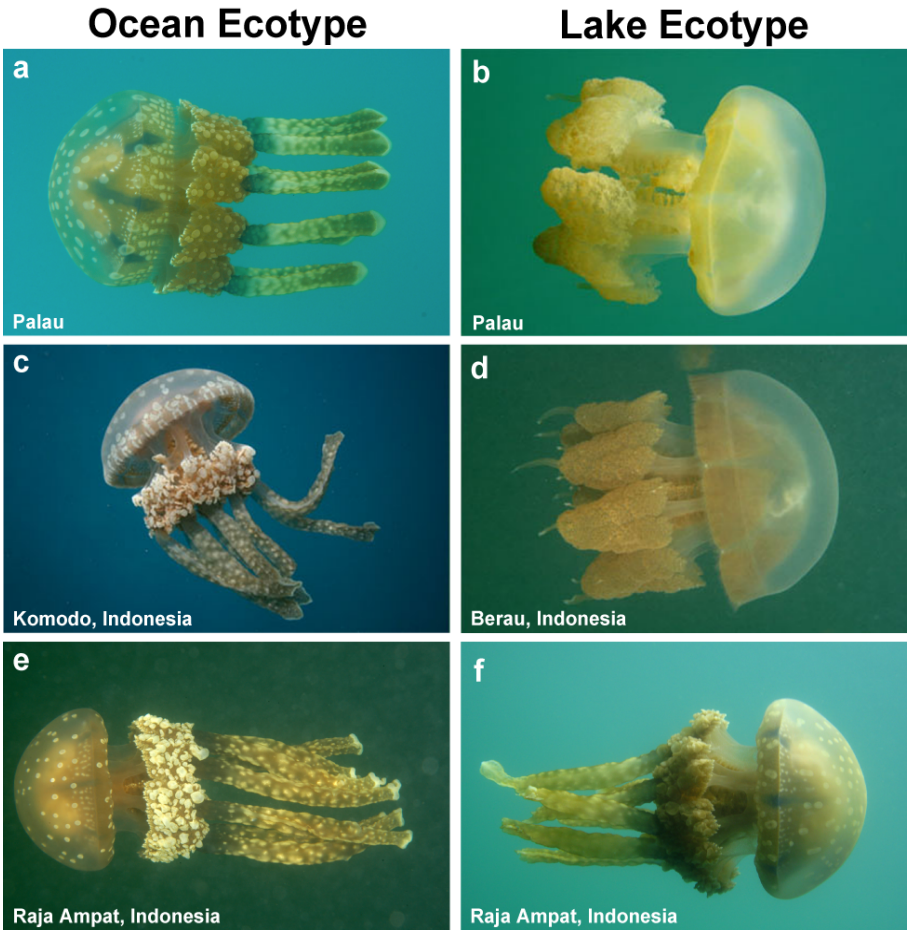

Figure 1. *Mastigias* occurs in two ecotypes across the Indo-West Pacific. The left column shows individuals with the ocean ecotype, whereas the right shows individuals from marine lakes. The ocean ecotype is similar across regions, whereas the similarity of the lake ecotype depends both on the habitat and the physical isolation of the lake from the ocean. Similarity between regions within an ecotype may be crypsis. *Mastigias* from **(a)** Ngerchaol Cove, Ngerchaol, Palau, a semi-enclosed ocean site that experiences full tidal exchange with the surrounding lagoon, **(b)** from Ongeim'l Tketau, Mecherchar, Palau, a meromictic lake that is hydrographically isolated from the ocean and formed approximately 10,000 years before present, **(c)** coastal waters surrounding Komodo, Indonesia (photo courtesy of R. Olsen), **(d)** Haji Buang, Berau province, Indonesia, a meromictic lake possibly younger in approximate age (based on depth) and less isolated (based on tidal dampening relative to the ocean) than OTM (Hamner and Hamner, 1998; Becking et al., 2011; Dawson, unpublished; photo courtesy of L. Bell), **(e)** coastal waters surrounding Waigeo, West Papua, Indonesia, and **(f)** Lake A, Gam, West Papua, Indonesia, a shallow and holomictic lake with high tidal exchange. The 'typical' lake ecotype, as seen in (b) and (d) also occurs in at least one lake in Misool, West Papua, Indonesia (Becking et al., 2015; Dawson et al., 2009), but is not shown because specimens were not available for this study.

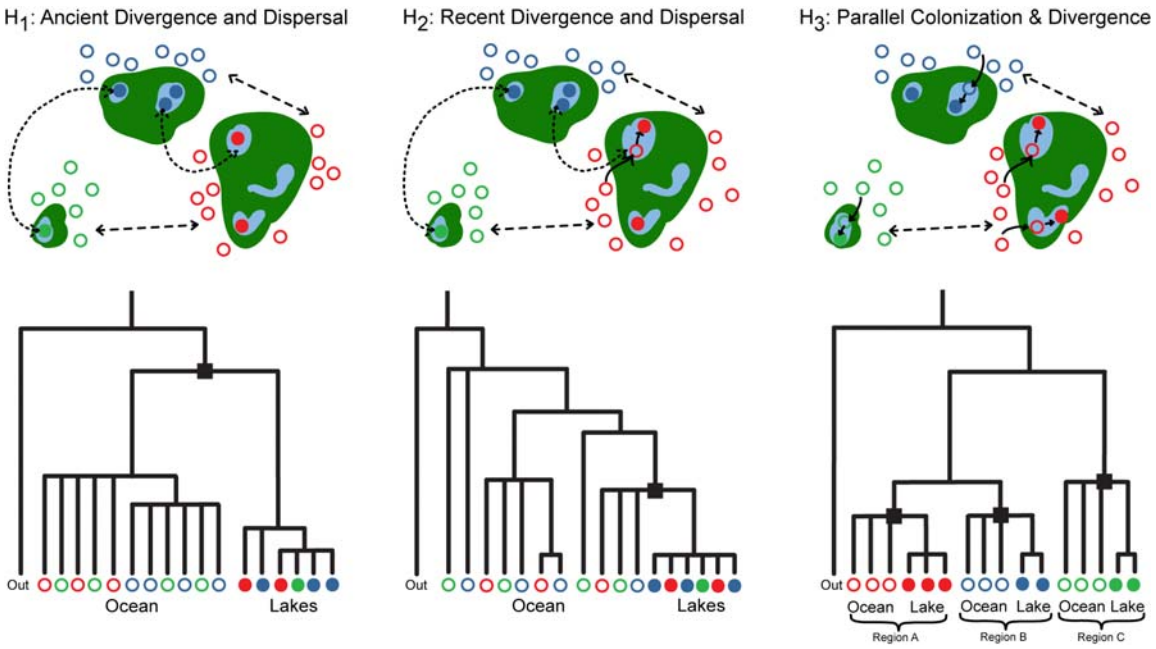

Figure 2. The pattern of *Mastigias* morphology seen across the IWP may be explained by one of three evolutionary hypotheses. **H_1** shows an ancient divergence between lake and ocean ecotype clades, and gene flow between regions within each ecotype. In this scenario, the Last Glacial Maximum (LGM) would occur after the divergent event between ecotypes. **H_2** indicates a eurymictic ocean population with one recent colonization event into a lake and subsequent divergence therein. The LGM would have occurred prior to the divergent event in this scenario. In both H_1 and H_2, regional phylogeographic structure may exist within ecotypes although this is not depicted. **H_3** depicts regional isolation in ocean ecotypes and multiple colonization events in each region from coastal ocean waters into marine lakes. The LGM would have occurred prior to the divergent event in this scenario. For each hypothesis, a schematic of proposed geographic pattern of evolution is on top and the resulting phylogenetic hypothesis is below. Different hues denote different regions. Open circles denote ocean ecotypes; solid circles, lake ecotypes. Dashed lines indicate dispersal between ocean sites; dotted lines dispersal between lakes (via the ocean). Solid lines indicate a post-glacial colonization event from the ocean to a lake and subsequent divergence. Black squares on phylogenetic trees indicate the node in which the most recent common ancestor between lake and ocean ecotypes occurs.

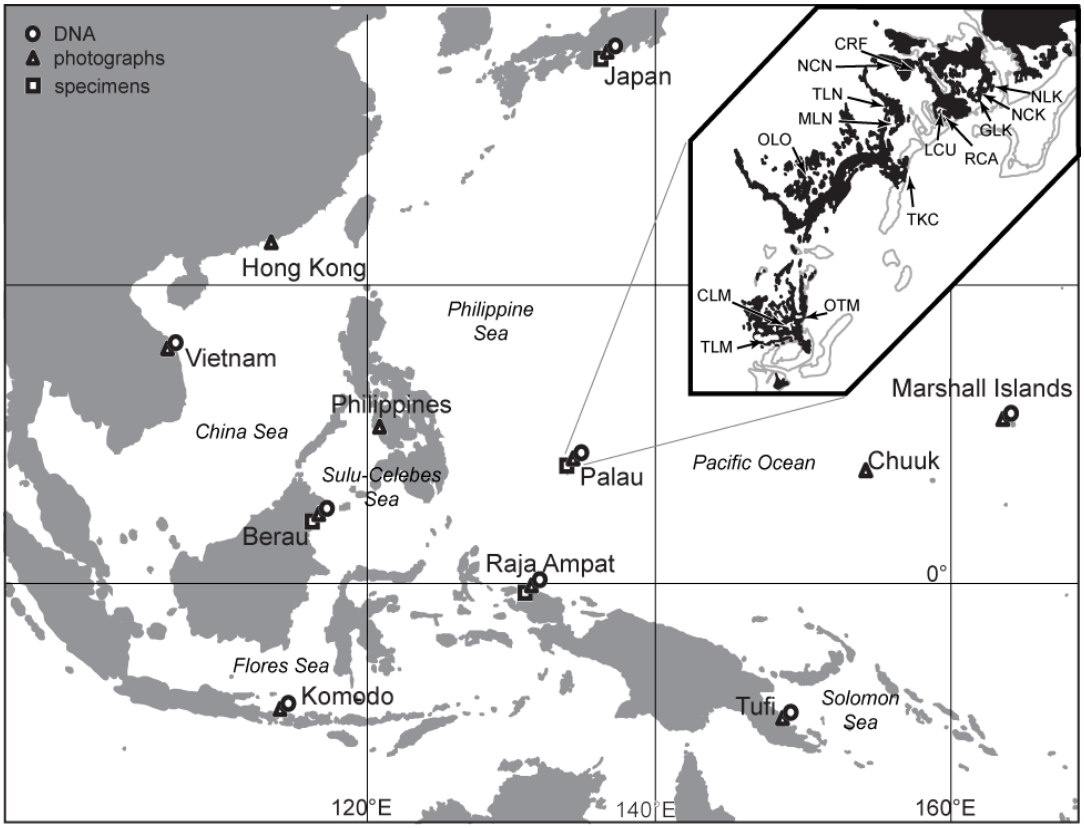

Figure 3. Map of the Indo-West Pacific showing sampled locations. Symbols denote the sample type available from each location. The exact location for the photo from the Philippines is not known. The inset shows the fourteen locations in Palau used in phylogeographic analyses. Details of location and sample sizes for each data type are in Table 1; samples labeled as "Lagoon" in Table 1 are not shown as they came from two sites within the ocean lagoon, one near CLM and the other near OTM.

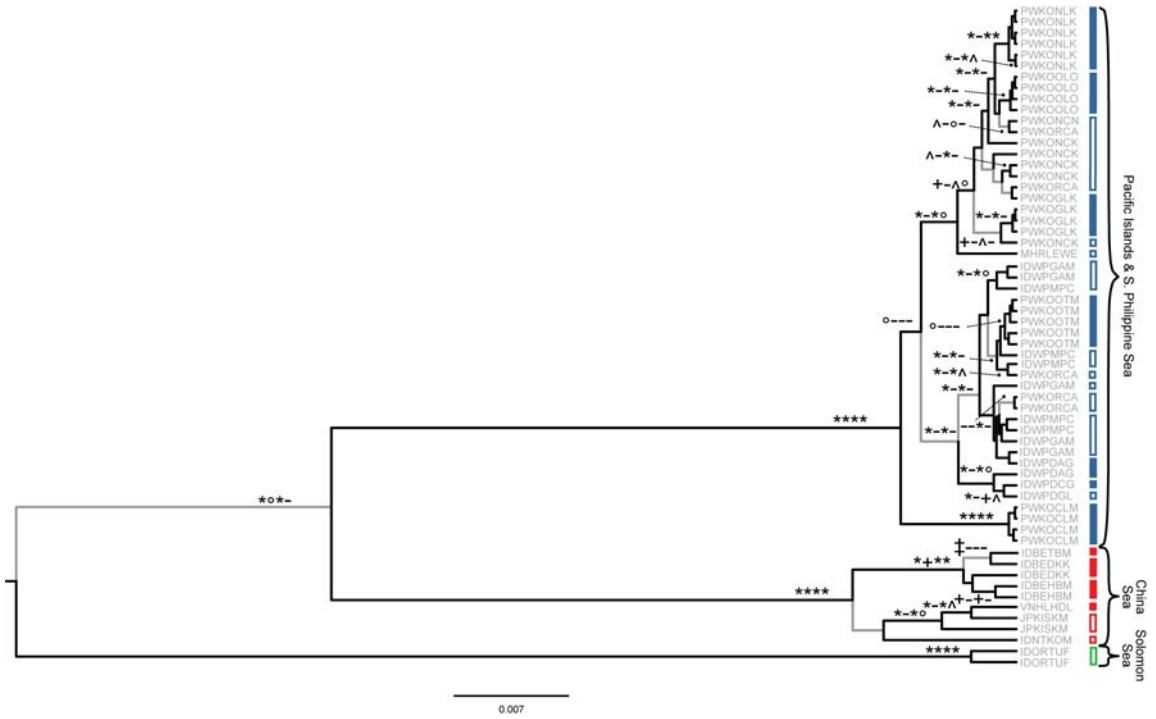

Figure 4. Bayesian species tree constructed via tree congruence of gene trees. Branches are labeled with support indices Bayesian posterior probability (BPP; as a percentage) and maximum likelihood bootstrap percentage (MLB) in the following order: species tree of all loci BPP first, then the BPP species tree of all specimens, followed by the BPP/MLB from the Bayesian and maximum likelihood partitioned species tree. Symbols for support values are: * ≥ 95, 95 > + ≥ 90, 90 > ^ ≥ 80, 80 > ° ≥ 70, all other values < 70 are marked with a hyphen; a ‡ indicates support not applicable due to missing specimens in that tree. Dotted lines connect the branch and its support values if the latter could not fit in the space above the branch. Grey branches appeared in Bayesian trees but not in the Maximum Likelihood tree. Vertical bars at the tips indicate environment type and major clade: open bars are ocean locations, filled bars are lakes, colors correspond to major clades given by brackets.

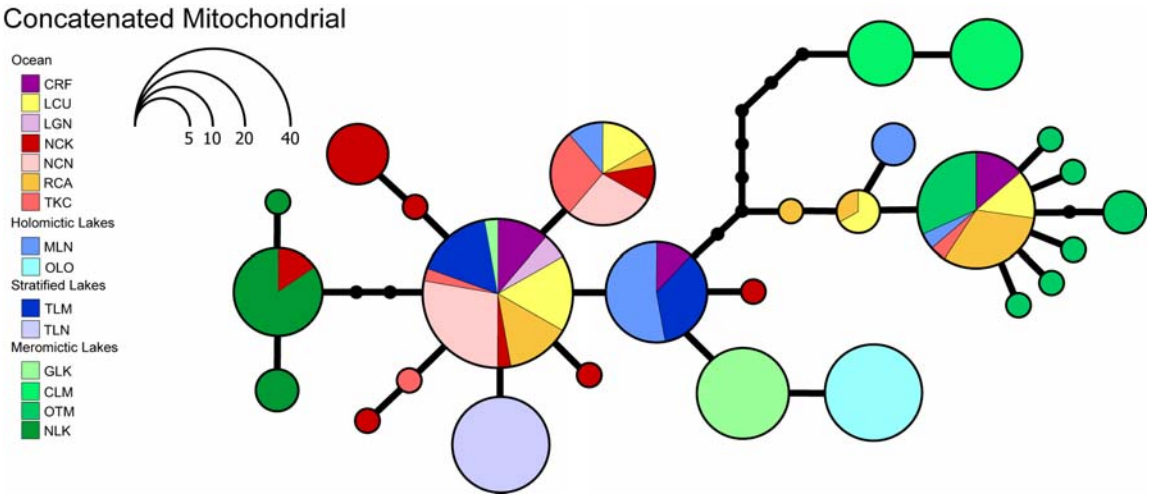

Figure 5. Haplotype network of concatenated COI and COIII sequences for locations in Palau. Each branch corresponds to one nucleotide difference; unsampled haplotypes are indicated by a black dot. Circle area is proportional to the number of individuals with that haplotype, as shown by the scale in the center. Sites are identified by the last three letters of site codes provided in Table 1; colors indicate the locations at which individuals were sampled. Ocean locations are denoted by warm colors, lakes by cool colors and two well-connected lakes by the brighter blue hues.

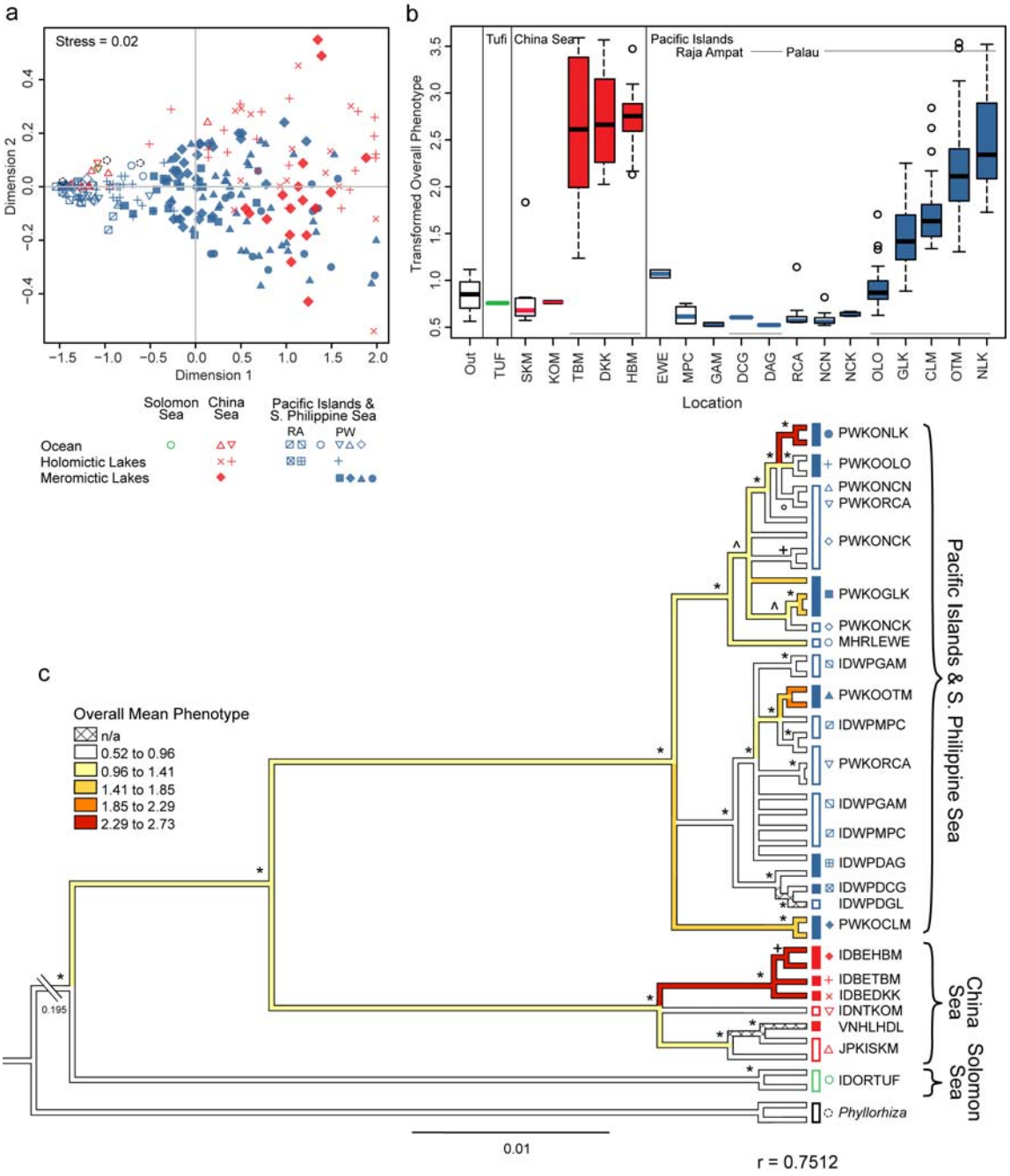

Figure 6. Morphological variation of *Mastigias* across the IWP. **(a)** nMDS analysis of the *IWP2* data matrix. Symbols used in the plot are shown with site acronyms in the Bayesian species tree in (c); full site details are given in Table 1. Specimens from the Pacific Islands & South Philippine Sea (PISPS) clade are in blue, China Sea in red and Solomon Sea in light green. Environment type and clades for each symbol are given below the nMDS plot, with ocean locations designated by open, holomictic lakes by crossed and meromictic lakes by closed symbols. Within the PISPS clade, further

distinction is made for specimens from Raja Ampat (RA) and Palau (PW). **(b)** Linearized overall phenotypic similarity. Boxes enclose the inner quartile range of values with the heavy line denoting the median. Dashed lines extend to the minimum and maximum values, excepting outliers indicated by open circles. Open boxes are from ocean locations; shaded boxes from lakes; lakes are additionally denoted by a line above the x asis. Hue indicates corresponding clade. Lakes are ordered from left to right by increasing depth, which is a proxy for duration of isolation (per Appendix IV). **(c)** Bayesian species tree with posterior probability as a percentage for each node given as * ≥ 95, 95 > + ≥ 90, 90 > ^ ≥ 80, and 80 > ° ≥ 70; branches with less than 70% posterior probability in the species tree were collapsed. Only enough tips from each location to preserve the topology are shown. The double slash at the terminus of the ingroup indicates the distance has been truncated; the full distance of the branch is given below. Shades indicate the mean distance on the nMDS from (minX, minY), and should be interpreted only in that light colors are similarly close to (minX, minY); whereas dark values are far from (minX, minY). Shades at the tip are the states assigned to the tree; other shading of internal branches indicates maximum parsimony ancestral state character reconstruction. Vertical bars adjacent to leaves indicate the environment type and major clade; open bars are ocean locations, shaded bars are lakes; hues correspond to clades given in brackets. Phylogenetically independent contrasts show overall phenotypic similarity is correlated with environment ($r = 0.7956$, $p << 0.0001$) after adjusting the degrees of freedom for soft polytomies.

Appendix I. Primers used in this study. H3 primers denoted with a * were designed for H3a on specimens from Palau, specifically. References with a [1] indicate the original location of the primer and [2] indicate the location of the specific thermocycle used to amplify jellyfish.

Region	Primer Name	Sequence (5' to 3')	Primer Pairs	Thermocycle	Reference
COI	LCOjf	GGTCAACAAATCATAAAGATATTGGAAC	a, b, c		Dawson and Hamner, 2005
	MpHCO	CAAAATAGATGCTGATACAAAATAG	a	94°C 8 min, 51°C 2 min, 72°C 2 min, 94°C 4 min, 52°C 2 min, 72°C 2 min, [94°C 45 s, 53°C 45 s, 72°C 60 s] x 33, 72°C 10 min, 4°C end	Dawson and Hamner, 2005[1]; Dawson, 2005[2]
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	b	as in (a)	Folmer et al., 1994[1]; Dawson, 2005[2]
	Aa_HCOI_12582	AGCAGGGTCGAAGAAAGATGTATT	c	94°C 3 min, [94°C 30 s, 53°C 60 s, 72°C 75 s] x 35, 72°C 10 min, 4°C end	Bayha and Dawson, 2010
16S	16S-L	GACTGTTTACCAAAAACATA	d		Ender and Schierwater, 2003
	Aa_H16S_15141H	AGATTTTAATGGTCGAACAGAC	d	94°C 3 min, [92°C 45 s, 50°C 60 s, 72°C 90 s] x 38, 72°C 10 min, 4°C end	Bayha and Dawson, 2010
H3	Histone 3 F Deg	ATGGCTCGTACCAAGCAGACVGC	e		Colgan et al., 1998
	Histone 3 R Deg	ATATCCTTRGGCATRATRGTGAC	e	94°C 5 min, [94°C 30 s, 56°C 30 s, 72°C 60 s] x 35, 72°C 10 min, 4°C end	Colgan et al., 1998[1]; this study[2]
	H3_Mp_9F*	CGACCGAGGAAAGGCACCC	f		This study
	H3_Mp_327R*	CGTTTTGCGTGGATGGCGC	f	94°C 3 min, [94°C 30 s, 54°C 30 s, 72°C 60 s] x 3, [94°C 30 s, 50°C 30 s, 72°C 60 s] x 32, 72 10 min, 4°C end	This study
COIII	COIIIA	ATTTAGTTGATCCTAGGCCTTGACC	g		O'Foighil, unpublished
	COIIIB	ACTCAAACCACATCTACAAAATG	g	94°C 5 min, [94°C 45 s, 50°C 60 s, 72°C 75 s] x 39, 72°C 10 min, 4°C end	O'Foighil, unpublished[1]; Bayha et al., in review[2]
	COIII_Mp_6670Fb	ACGTAGCGTCGTGTGGAAC	h, i		This study
	COIII_Mp_7339Rdeg	ATAYCAACTGGCTGCYTCAAACC	h	94°C 5 min, [94°C 45 s, 56°C 60 s, 72°C 75 s] x 39, 72°C 10 min, 4°C end	This study
	COIII_Mp_7339R	ATACCAACTGGCTGCCTCAAACC	i	94°C 5 min, [94°C 45 s, 56°C 60 s, 72°C 75 s] x 39, 72°C 10 min, 4°C end	This study

Appendix I References

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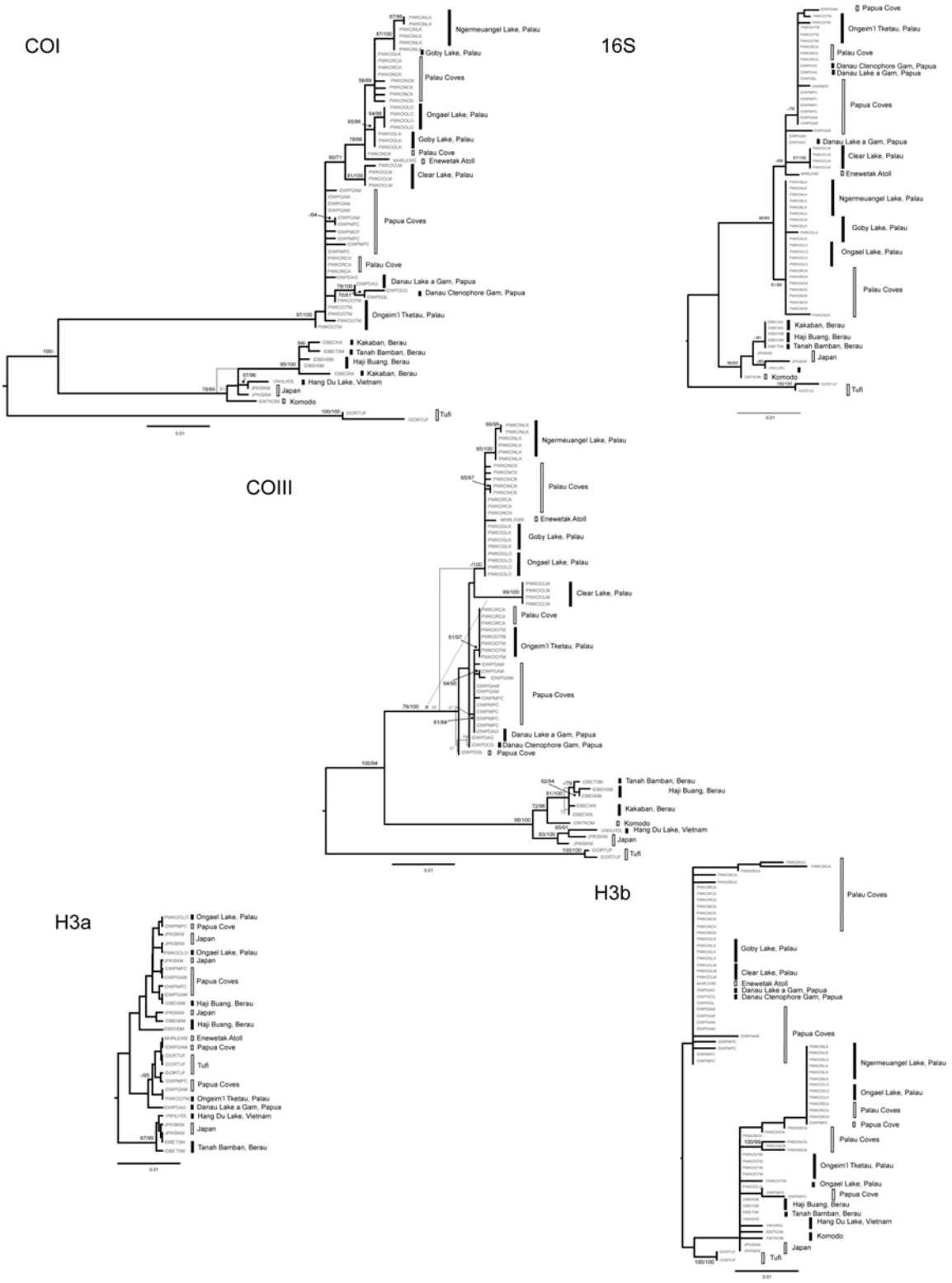
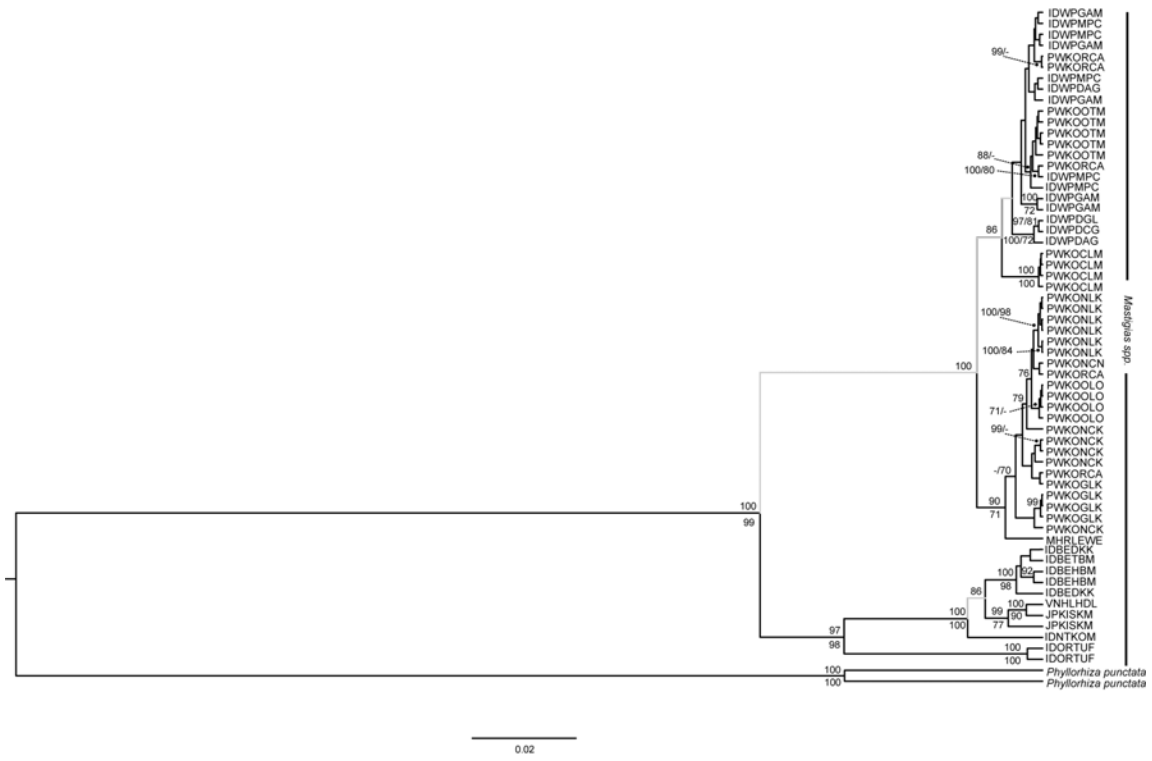
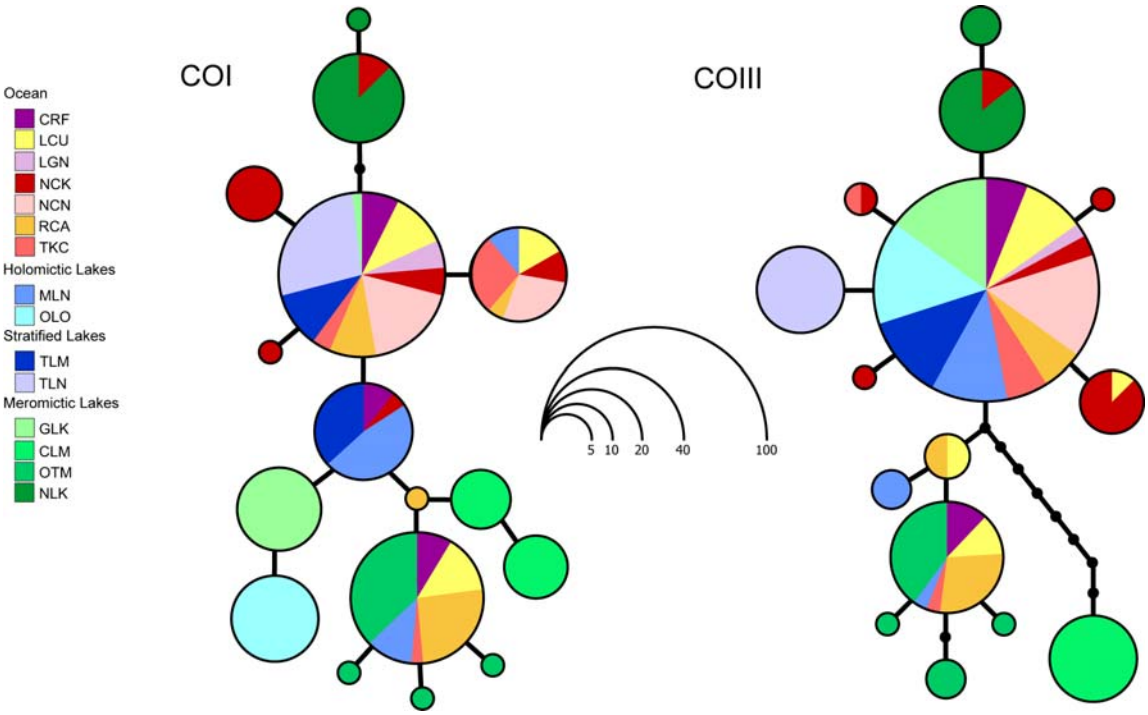

COI
Ngermeuangel Lake, Palau
Goby Lake, Palau
Palau Coves
Ongael Lake, Palau
Goby Lake, Palau
Palau Cove
Enewetak Atoll
Clear Lake, Palau
Papua Coves
Palau Cove
Danau Lake a Gam, Papua
Danau Ctenophore Gam, Papua
Ongeim'l Tketau, Palau
Kakaban, Berau
Tanah Bamban, Berau
Haji Buang, Berau
Kakaban, Berau
Hang Du Lake, Vietnam
Japan
Komodo
Tufi
16S
Papua Cove
Ongeim'l Tketau, Palau
Palau Cove
Danau Ctenophore Gam, Papua
Danau Lake a Gam, Papua
Papua Coves
Danau Lake a Gam, Papua
Clear Lake, Palau
Enewetak Atoll
Ngermeuangel Lake, Palau
Goby Lake, Palau
Ongael Lake, Palau
Palau Coves
Kakaban, Berau
Haji Buang, Berau
Tanah Bamban, Berau
Japan
Komodo
Tufi
COIII
Ngermeuangel Lake, Palau
Palau Coves
Enewetak Atoll
Goby Lake, Palau
Ongael Lake, Palau
Clear Lake, Palau
Palau Cove
Ongeim'l Tketau, Palau
Papua Coves
Danau Lake a Gam, Papua
Danau Ctenophore Gam, Papua
Papua Cove
Tanah Bamban, Berau
Haji Buang, Berau
Kakaban, Berau
Komodo
Hang Du Lake, Vietnam
Japan
Tufi
H3a
Ongael Lake, Palau
Papua Cove
Japan
Ongael Lake, Palau
Japan
Papua Coves
Haji Buang, Berau
Japan
Haji Buang, Berau
Enewetak Atoll
Papua Cove
Tufi
Tufi
Papua Coves
Ongeim'l Tketau, Palau
Danau Lake a Gam, Papua
Hang Du Lake, Vietnam
Japan
Tanah Bamban, Berau
H3b
Palau Coves
Goby Lake, Palau
Clear Lake, Palau
Enewetak Atoll
Danau Lake a Gam, Papua
Danau Ctenophore Gam, Papua
Papua Coves
Ngermeuangel Lake, Palau
Ongael Lake, Palau
Palau Coves
Papua Cove
Palau Coves
Ongeim'l Tketau, Palau
Ongael Lake, Palau
Papua Cove
Haji Buang, Berau
Tanah Bamban, Berau
Hang Du Lake, Vietnam
Komodo
Japan
Tufi

Appendix II. Maximum Likelihood gene trees for COI, 16S, COIII, H3a and H3b. Values represent bootstrap value percentage / Bayesian posterior probability as a percentage above 60. A dash means the branch was not supported in the given tree. Any discrepancies between the Maximum Likelihood or Bayesian trees are shown with the Bayesian topology in grey. These trees are shown without the outgroup, but are rooted at the position where the node to the outgroup would be when *Phyllorhiza* is included.



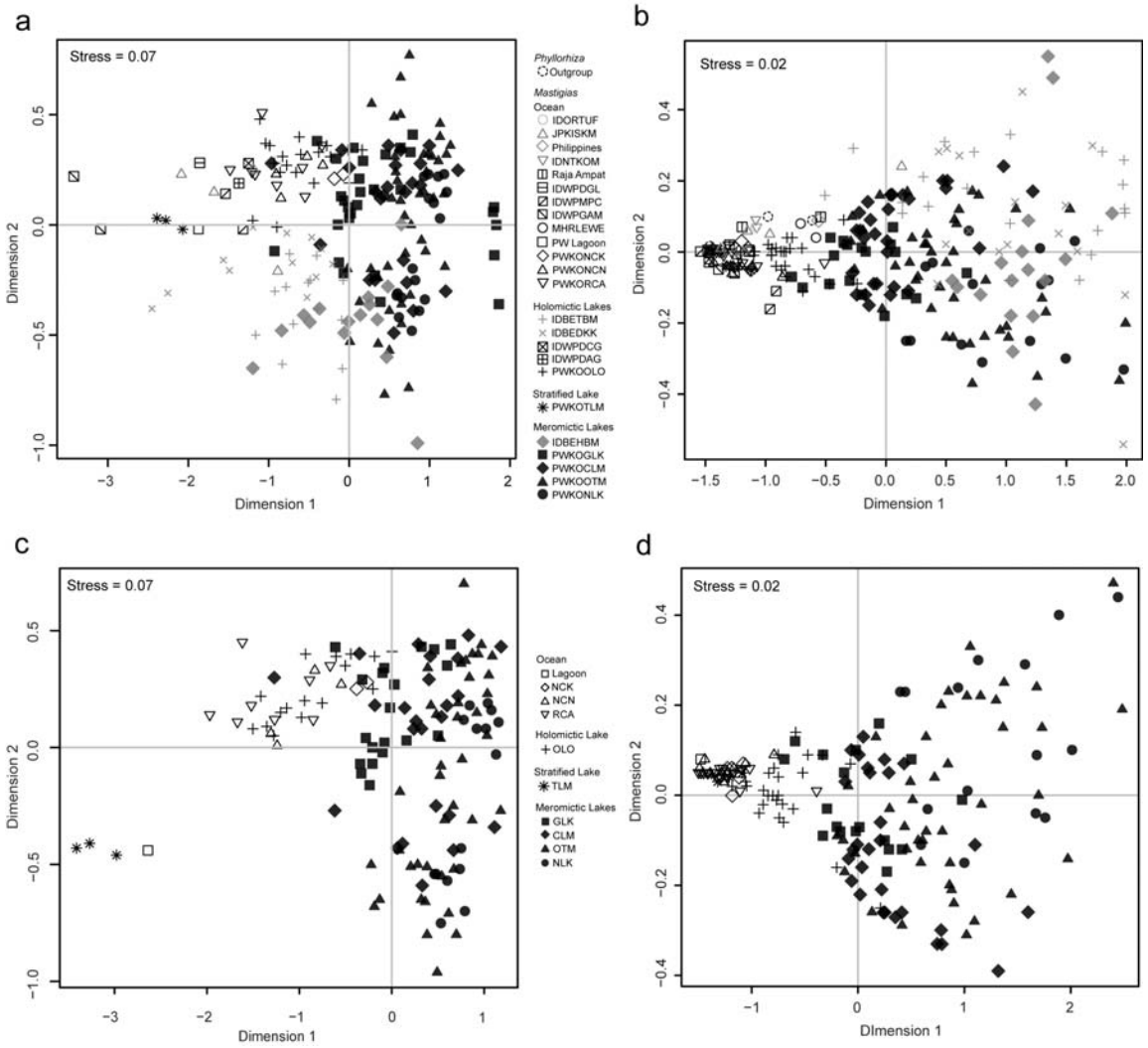
Appendix III. Partitioned Bayesian species tree from COI, COIII, 16S, H3a and H3b gene trees, including *Phyllorhiza punctata* as the outgroup. Branches are labeled with support indices BPP/MLB where MLB is maximum likelihood bootstrap percentage and BPP Bayesian posterior probability as a percentage. Only support ≥70 BPP or MLB are shown. Grey branches are those that did not occur on the Maximum Likelihood tree.



Appendix IV. Haplotype networks of COI and COIII in Palau. Each branch corresponds to one nucleotide difference; unsampled haplotypes are indicated by a black dot. Circle size is proportional to the number of individuals sampled which had that haplotype; the scale in the center gives an idea of the relative sizes. Colors correspond to locations at which the individuals sampled were taken. Ocean locations are denoted by warm colors, holomictic and 'stratified' (stratified, but not meromictic) lakes by blues and meromictic lakes by greens. The networks show a common, central haplotype found in ocean locations and some holomictic lakes. Most ocean locations have a greater diversity of haplotypes than any one lake

Appendix V. Matrix of the Φ_{ST} values between Palau locations; numbers of individuals are given in Table 1. ^{ns} = not significantly different from zero. Ocean locations and most holomictic lakes pairings are not significantly different from zero and indicate a genetically mixed, or for TLN, recently isolated population. Meromictic lakes and OLO have high values of isolation from all locations indicating independent isolation. Φ_{ST} values from a randomly subsampled dataset of n = 9 for all locations except TKC (n = 8) shows the same pattern.

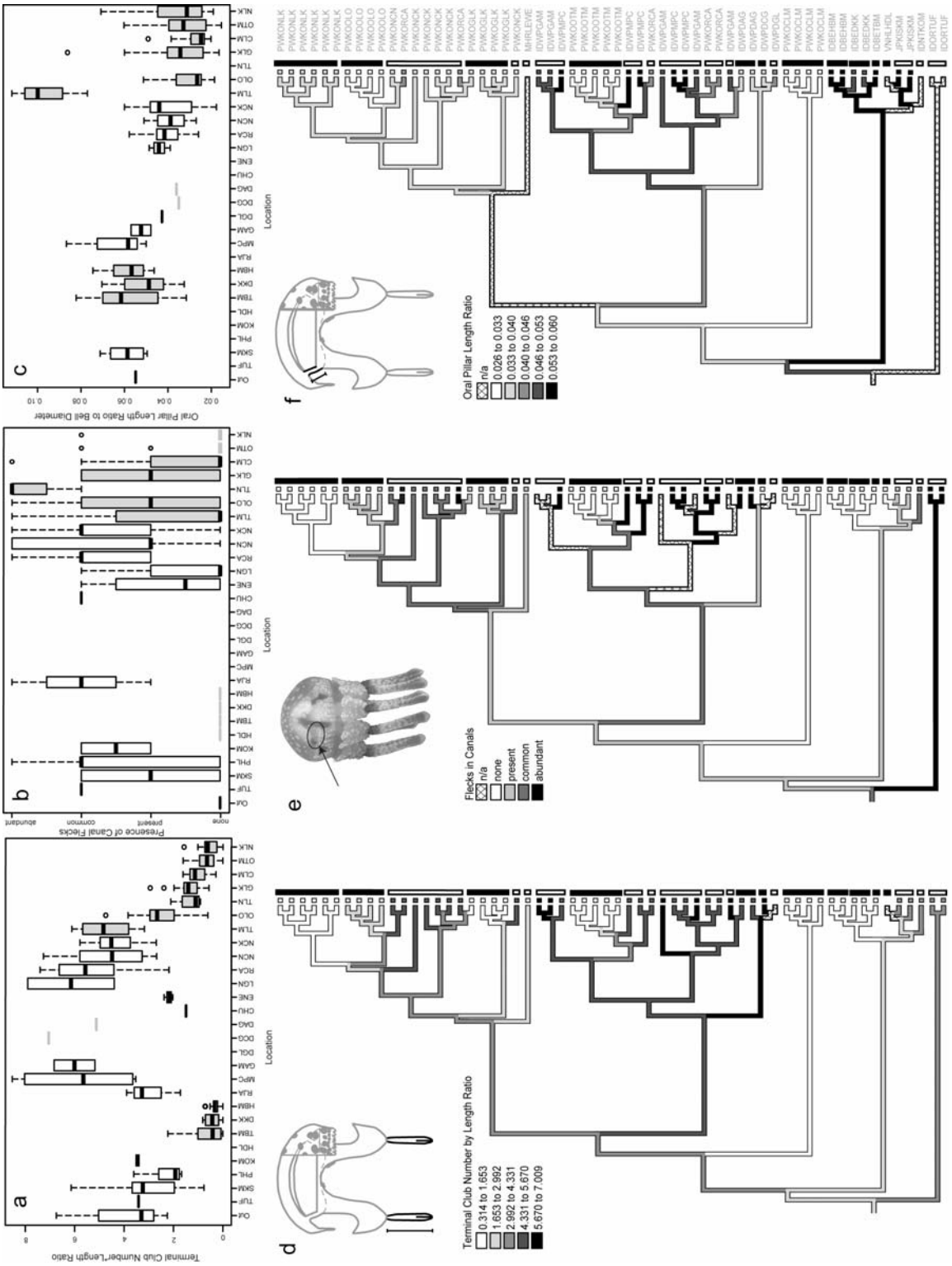
Body of Water Type		Ocean						Holomictic Lakes		Stratified Lakes		Meromictic Lakes		
		RCA	LCU	CRF	NCK	NCN	TKC	MLN	OLO	TLM	TLN	GLK	CLM	OTM
Ocean	LCU	0.040^{ns}												
	CRF	0.005^{ns}	-0.067^{ns}											
	NCK	0.432	0.24	0.268										
	NCN	0.512	0.253^{ns}	0.372	0.196									
	TKC	0.297^{ns}	0.055^{ns}	0.137^{ns}	0.168^{ns}	0.078^{ns}								
Holomictic Lakes	MLN	0.141^{ns}	0.039^{ns}	-0.013^{ns}	0.31	0.377	0.189^{ns}							
	OLO	0.679	0.629	0.68	0.706	0.929	0.79	0.603						
Stratified Lakes	TLM	0.431^{ns}	0.204^{ns}	0.214^{ns}	0.23	0.389	0.276	0.172^{ns}	0.903					
	TLN	0.628	0.494	0.59	0.505	0.821	0.629	0.574	1	0.837				
Meromictic Lakes	GLK	0.581	0.484	0.515	0.573	0.831	0.653	0.415	0.882	0.726	0.953			
	CLM	0.832	0.844	0.871	0.893	0.964	0.914	0.859	0.982	0.959	0.982	0.97		
	OTM	0.326	0.554	0.563	0.783	0.887	0.765	0.619	0.923	0.863	0.923	0.895	0.929	
	NLK	0.71	0.65	0.708	0.621	0.868	0.748	0.698	0.962	0.865	0.944	0.928	0.97	0.917



Appendix VI. nMDS plots showing differences in locations within **a, b)** the IWP and **c, d)** Palau scored on the morphological data matrix; matrices for each nMDS are: **a)** *IWP1*, **b)** *IWP2*, **c)** *PW1* and **d)** *PW2*. Analyses show the same pattern given a large number of individuals with a few characters **(right)** or a smaller number of individuals with more characters **(left)** and are statistically similar. Shade denotes clade: light grey = Solomon Sea, medium grey = China Sea, black = Pacific Islands. Dashed symbols are for the outgroup, *Phyllorhiza*. Open symbols are ocean locations, lined symbols are holomictic lakes, the star symbol is for the 'stratified' lake and filled symbols are meromictic lakes. Individual locations are as given in the legend. Specimens collected from specific locations have a seven letter code (reference Table 1), whereas specimens without a specific site (i.e., were from photographs and only identified to region) have regions listed; for the Palau plots in **c)** and **d)**, the prefix "PWKO" was left off location codes in the legend for clarity. Palau samples labeled as "Lagoon" in came from two sites within the ocean lagoon, one near CLM and the other near OTM.

Appendix VII. Pair-wise post-hoc Tukey test comparisons of Palau locations on the *PW2* nMDS for Dimension 1 (bottom triangle) and Dimension 2 (top triangle). Significant pair-wise comparisons after Sequential Bonferroni corrections are denoted with an *. Most differentiation between locations is on Dimension 1, as means for locations in Dimension 2 are statistically insignificant even as the variation within locations increases. Most significant comparisons are between meromictic lakes and ocean sites (15/16) or holomictic and 'stratified' lakes (7/8). Holomictic and 'stratified' lakes and ocean sites are statistically indistinguishable.

		Ocean				Holomictic Lake	Stratified Lake	Meromictic Lakes			
		Lgn	NCN	NCK	RCA	OLO	TLM	GLK	CLM	OTM	NLK
Ocean	Lgn										
	NCN										
	NCK										
	RCA										
Holomictic Lake	OLO										
Stratified Lake	TLM										
Meromictic Lakes	GLK		*	*	*	*					
	CLM	*	*	*	*	*	*				*
	OTM	*	*	*	*	*	*	*	*		
	NLK	*	*	*	*	*	*	*	*		


a
Terminal Club Number/Length Ratio
b
Presence of Canal Flecks
none
present
common
abundant
c
Oral Pillar Length Ratio to Bell Diameter
Location
d
Terminal Club Number by Length Ratio
0.314 to 1.653
1.653 to 2.992
2.992 to 4.331
4.331 to 5.670
5.670 to 7.009
e
Flecks in Canals
n/a
none
present
common
abundant
f
Oral Pillar Length Ratio
n/a
0.026 to 0.033
0.033 to 0.040
0.040 to 0.046
0.046 to 0.053
0.053 to 0.060

Appendix VIII. Morphological variation in *Mastigias* shows a similar pattern of ocean vs. lake ecotype across morphological characters. Boxplots show the variation in a given character for each location. Ocean locations are open boxes and lakes shaded grey. Below each boxplot is the corresponding phylogenetic contrast on the Bayesian species tree. Colors on the tree branches are coded by the legend to the left of each tree. Vertical bars at the end of the branches indicate the habitat type: open bars are ocean sites, filled bars are lakes. The schematic indicates the character being measured: (a) and (d), terminal club length; (b) and (e), presence of flecks in the canals; (c) and (f), oral pillar length. Terminal club length and the presence of flecks in the canals show the typical lake/ocean dichotomy. The oral pillar length shows a dichotomy in region, not ecotype.

CHAPTER 3:

ENVIRONMENTAL AND DENSITY RELATED VARIATIONS IN MORPHOLOGY AND BEHAVIOR DESPITE GENETIC SIMILARITY IN THE SCYPHOZOAN JELLYFISH *MASTIGIAS PAPUA*

1 Abstract

Spatio-temporal environmental variation can produce phenotypic variation among populations, which may alter their evolutionary trajectories in the future. Efforts to understand the consequences of environmental variation for scyphozoans have produced mixed results, potentially due to the challenge of delimiting populations and documenting population dynamics in open pelagic marine systems. Marine lakes, bodies of seawater entirely surrounded by land, provide an opportunity to study discrete populations of medusae and capture fine-scale dynamics and responses to perturbations. I compared *Mastigias papua* medusae sampled before and after a perturbation event, that consisted of a medusa population crash concurrent with a stratified water layer of increased temperature and salinity, in Ngermeuangel Lake, Palau. I reconstructed intraspecific genetic relationships, measured variation in up to 40 morphological characters, and assessed three different metrics of behavior to evaluate three hypotheses for the source of the variation: recolonization, differential genotype expression, and plasticity. I found significant differences between demes in overall morphology ($p < 0.001$) and 24 of 45 individual characters ($p < 0.001$), and in two of the three behavioral metrics, swimming speed and pulse rate ($p < 0.005$), despite finding no significant variation in mtDNA between demes ($\Phi_{ST} = 0.03$, $p = 0.09$). Thus I reject the hypothesis of recolonization from the ocean, as the medusae upon recovery were genetically similar to the prior deme, and significantly different from any of the potential source locations ($\Phi_{ST} > 0.48$, $p > 0.001$). However, I could not distinguish between the other two hypotheses. Either differential genotypic expression or plasticity could alter the evolutionary trajectory of the population as it recovers. The phenotypic variation of *Mastigias* in response to a perturbation, and its potential future effects, illustrates that, to understand scyphozoan responses to climatic variability, meta-analyses of population studies are needed.

2 Introduction

Intraspecific variation is a fundamental part of evolution, as genetically-based phenotypic variation in populations is the raw material for speciation (Mayr 1963, Endler 1986). Moreover, intraspecific phenotypic variation can underlie patterns in the abundance, distribution, and ecological roles of, and the interactions between, organisms (Grant et al. 1976, Grant & Grant 1980, Sanford et al. 2003, Gilman 2005, Ezard et al. 2009, McClellan et al. 2010, Bolnick et al. 2011, Cubillo et al. 2012, Chiaverano et al.

2016), including responses to disturbances (Berlow & Navarrete 1997, Schoener et al. 2001, Davis et al. 2005, Visser 2008, Emery & Ackerly 2014). Marine populations exist in a heterogeneous environment that can vary spatio-temporally along scales from seconds and meters to decades and latitudes (Barry & Dayton 1991). This environmental variability has produced a wide array of morphological, behavioral, and life-history traits between and within species (Hadfield & Strathmann 1996, Barry & Dayton 1991). Intraspecific variation in morphology and behavior are of evolutionary importance especially during extreme environmental perturbations, which can shift selection landscapes (Boag & Grant 1981, Gibbs & Grant 1987a, b, Price et al. 2003, Pelletier et al. 2009).

Sources of intraspecific variation in morphology and behavior can be phenotypic, genetic, or a combination of phenotypic and genetic processes. Local environmental cues can precipitate plastic phenotypic responses that result in variation across a species' distribution (Chiaverano et al. 2016) or in a single location at different times (Pocock 1912). Spatio-temporal variation also can be a result of heterogeneous distribution of genotypes (Dawson & Hamner 2003, Dawson 2005a), or differential fitness of particular genotypes under varied environmental conditions (Grant et al. 1976, Magalhaes et al. 2009). There can also be significant interactions between genotypes and environment, producing genetically based, varying plastic responses to environmental signals (Via & Lande 1985). These sources of variation can facilitate adaptive evolution during local environmental perturbations.

An increasing frequency of extreme disturbances and climatic variability (Harley et al. 2006, IPCC 2007, Jentsch et al. 2007, Brierley & Kingsford 2009) also increases the need for understanding population responses to environmental variation. Greater understanding is needed in heterogeneous marine environments, where population dynamics are often little understood and species' ranges poorly delimited (Palumbi 1994, Carr et al. 2003, Bowen et al. 2013), especially for organisms which inhabit open, pelagic systems, such as pelagic predatory scyphomedusae. Medusa populations are highly dynamic and capable of aggregative behavior that can exert considerable trophic pressure (Graham et al. 2001, Purcell 2005). Yet even though scyphomedusae are important pelagic predators (Purcell 2005), little is conclusively known about scyphozoan responses to environmental variation; the resulting dearth of knowledge limits prediction of scyphozoan responses to environmental variability. Attempts by large spatial-scale studies to relate changes in abundance of medusozoans to inter-annual anomalies in climate indices have produced mixed results (Brotz et al. 2012, Condon et al. 2013), potentially because populations' responses to climatic forcing vary locally and regionally (Dawson et al. 2001, Dawson et al. 2015).

Coarse temporal and geographic scales in studies of scyphomedusae are largely due to the challenges of documenting pelagic population dynamics in open systems. Marine lakes – bodies of seawater entirely surrounded by land – provide an opportunity to study discrete jellyfish populations (Dawson & Hamner 2005; Dawson et al. 2015), capturing finer-scale dynamics and responses to perturbations of the system. *Mastigias* medusa populations in marine lakes are the result of repeated, independent colonization of the lakes from adjacent oceans (Swift et al. 2016). Subsequent parallel morphological evolution of lake medusae has resulted in a 'lake ecotype' morphology. *Mastigias*

medusae populations in marine lakes have tended to have spectacularly high population sizes, although also varying susceptibility to perturbations (Hamner & Hauri 1981, Dawson et al. 2001, Dawson et al. 2015), including major population crashes associated with environmental variability (Dawson et al. 2001, Martin et al. 2006).

One such population crash occurred in Ngermeuangel Lake (NLK) in 2011, when the population of ~4-5 million medusae (Hamner & Hauri 1981, Martin 1999, Dawson et al. 2001) declined to zero within a few months. The medusae population decline coincided with a strongly stratified, high-salinity warm layer (35-37°C, 34-37psu) in the mixolimnion (the top habitable layer in the lake). As the medusae population started to recover, the morphology of the medusae differed from the morphology typically seen in NLK (Figure 1; Dawson 2005a, b). Changes in morphology and behavior during or after an environmental perturbation could be due to (1) colonization of the lake from the ocean following the medusae die-off, (2) a rare genotype in the benthic polyp population that strobilates in higher proportion under these conditions (and not at other times), or (3) phenotypic plasticity. Previous work has described the variation in the high density population (Dawson & Hamner 2003, Dawson 2005a, Dawson & Hamner 2005, Swift et al. 2016). I repeat those analyses on the low density medusa population to quantify any changes in (a) morphology, (b) swimming behavior, or (c) genetic composition, and (d) the most likely source(s) of the variation.

3 Methods

3.1 Study system

NLK is a meromictic (i.e. strongly stratified with an anoxic sulfide layer below the chemocline) marine lake in Palau, approximately 23 km from the renowned "Jellyfish Lake" (OTM). NLK formed approximately 12,000 years ago (Smittenberg et al. 2011) when rising seas flooded through cracks and fissures into the karst island; propagules of marine life were introduced to the lakes with the flooding seawater (Dawson et al. 2009). Contemporarily, NLK is essentially hydrodynamically and biologically isolated from the ocean (Hamner & Hamner 1998, Dawson & Hamner 2005). NLK is brackish, with an average salinity ~24psu, and a temperature profile which is fairly homogeneous at 32°C (Hamner & Hamner 1998).

Mastigias papua is a rhizostome jellyfish which harbors symbiotic *Symbiodinium*. *Mastigias* has a two part life cycle: a sexually reproducing pelagic medusa stage and an asexually reproducing benthic polyp stage. Polyps generally must be infected by *Symbiodinium* before they will strobilate and produce ephyrae (juvenile medusae) (Sugiura 1964, but see Dawson et al. 2001).

3.2 Genetic Analyses

Tissue samples of 38 specimens from the low density time period were collected for genetic analysis between August 2012 and August 2013, two to six specimens per

month depending on the standing population size. Samples were preserved in 95% EtOH and stored at -20°C. Tissue samples from the high density deme were collected during multiple field trips between 1996 - 2006, preserved in 95% EtOH and stored at -20°C. These specimens were added to sequence data from Dawson & Hamner (2005) and Swift et al. (2016) for a final specimen number at a time of high population density equal with the number of samples at low population density (Table 1).

DNA was extracted using a CTAB-phenol/chloroform protocol (Dawson & Jacobs 2001) and two mitochondrial markers were targeted for amplification: cytochrome *c* oxidase subunits I (COI) and III (COIII). COI was amplified using primer LCOjf (Dawson & Hamner 2005) paired with either MpCOI (Dawson & Hamner 2005), Aa_HCOI_12582 (Bayha & Dawson 2010), or HCO2198 (Folmer et al. 1994); COIII was amplified using COIII_Mp_6670b with COIII_Mp_7339R or COIII_Mp_733Rdeg (Swift et al. 2016). Polymerase chain reactions (PCRs) of 50 µl were composed of 0.5 µM primers, 5.0 µl 10x PCR buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 mM Bovine Serum Albumin (BSA), 0.1 µl Taq polymerase (Applied Biosystems, Foster City, CA), and 1 µl sample. Full or half reactions (of the same cocktail composition) were run on an Applied Biosystems 2720 thermal cycler. PCR reactions for COI using either MpCOI or HCO2198 included initial holds of 94°C for 8 minutes, 49-51°C for 2 minutes, 72°C for 2 minutes, 94°C for four minutes, 50-52°C for 1-2 minutes and 72°C for 1.5-2 minutes, followed by a cycle of denaturation at 94°C for 45 seconds, annealing between 51-53°C for 45 seconds and 72°C for 60 seconds cycled between 33-38 times, followed by a final extension step of 72°C for ten minutes before a final hold at 4°C. COI PCR reactions using Acro-HCO1-611 included an initial hold of 94°C for 3 minutes followed by thirty five cycles of a denaturation step at 94°C for 30 seconds, an annealing step of 53°C for 60 seconds and an extension step of 72°C for 75 seconds before a final extension step of 72°C for 10 minutes before a final hold at 4°C. PCR reactions for COIII had an initial hold at 94°C for 5 minutes followed by thirty nine cycles including a denaturation step of 94°C for 45 seconds, an annealing step of 52-56°C for 60 seconds and an extension step of 72°C for 75 seconds followed by a final extensions step of 72°C for 10 minutes before a final hold of 4°C. DNA was cleaned and sequenced at the University of California Berkeley's DNA Sequencing Facility.

Sequences were trimmed of primers, assembled into contigs, and visually inspected for base calls in Sequencher v. 4.1 (Gene Codes Corporation, Michigan, USA). COI and COIII were aligned in MUSCLE with a gap opening penalty of -1000 and a gap extension penalty of -100 and checked by eye. Any base position with missing information was excluded from further analysis. Parsimony haplotype networks of each locus independently, as well as concatenated, were constructed in TCS (Clement et al. 2000) and visualized in HapView (Salzburger et al. 2011). Population differentiation between the two time points was estimated by calculating Φ_{ST} in Arlequin v. 3.5.1.2 (Excoffier et al. 2005).

Additionally, population differentiation between the NLK low density deme and all cove locations was assessed. Sequences from cove locations were used from Swift et al. (2016). Since the sample size of those locations were n=15, fifteen samples were randomly selected from the NLK low density deme sequences for the comparison.

Population differentiation between the locations was estimated by calculating Φ_{ST} in Arlequin v. 3.5.1.2 (Excoffier et al. 2005).

3.3 Morphological Analyses

A total of 70 medusae were used for morphological analyses. Existing data from 19 high-density *Mastigias* (Dawson 2005a) were combined with new data from 36 preserved whole specimens and 23 *in situ* photographs of low-density *Mastigias* (Table 1). Whole-organism specimens were preserved in 4% formalin with seawater.

Morphological data from Dawson (2005a) targeted size classes, rather than a representative sample of the population. Thus, to describe any differences in medusae sizes, I compared bell diameters measured *in situ* of the high density population (Dawson & Hamner 2003) to those I measured *in situ* after photographing low-density individuals. All individuals measured *in situ* were measured to the nearest half centimeter. Overall mean and mean maximum bell diameters were compared between populations using ANOVAs. High density individuals were selected haphazardly, and given the large sample size (n=181), I believe are an accurate reflection of the medusae size range in the deme; low density individuals included all medusae seen at the time of sampling (n=25), and thus likely encapsulates the deme.

Morphological features described by Dawson (2005a) were measured on preserved specimens, except characters on coloration (*f1* to *f7*) which were scored from photographs since those characters are lost on preserved specimens. I did not include the characters mass or sex in this study, given the results of Dawson (2005a). Additionally, since the medusae in Dawson (2005a) were measured on unpreserved individuals, I tested for the possibility of differences due to shrinkage from preservation. I measured four individuals prior to and then after a 12 day preservation (Table1) and performed a t-tests for all characters (R. v. 2.11.1; R Development Core Team). No characters were significantly different after a Sequential Bonferroni correction (Rice 1989), and thus no corrective formula was applied before comparison between medusae deme morphologies.

Morphology was compared between demes using non-metric multidimensional scaling (nMDS) and principal component analysis (PCA) for an overall view of morphologies (as per Dawson 2005a), along with individual t-tests for each character to determine the underlying differences. Characters were normally distributed and had homogeneous variance.

The nMDS analysis was run on continuous features that were corrected for bell diameter, scaled and weighted, so as not to unduly emphasize redundant metrics, by the following procedure: 1) I calculated Spearman correlations between continuous features; significant correlations, after a table-wide Sequential Bonferroni correction (Rice 1989), were considered in subsequent steps; 2) I corrected for significantly different bell diameter distributions (Appendix I) by taking the ratio of bell diameter for any continuous feature that was significantly correlated with bell diameter in the initial Spearman correlation table; 3) Features were scaled by each feature's maximum value to range between 0 and 1, then 4) inversely down-weighted by a factor equal to the number of significant correlations plus the number of multiple measures in the dataset (i.e., bell thickness was measured in five different positions across a transect of the bell radius).

nMDS analysis on the subsequent data matrix was performed in Primer v. 5.2.9 (Clarke & Gorley 2001) using Euclidean distances. The nMDS analysis used ten randomly seeded starts to assess repeatability of the results (Clarke 1993). Minimum stress results were used to assess the data representation quality and a PERMANOVA calculation (R v. 2.11.1, adonis(), vegan package; Oksanen et al. 2011) used to determine if there was a significant difference in morphology due to density.

The PCA analyses were performed on categorical data that was scaled within the prcomp function in R (R. v. 2.11.1; R Development Core Team). PCA analyses were first performed with the characters which included blue pigmentation in the canals and bell and white pigmentation on the oral arm fringe, then without, as the two individuals displaying pigmentation in the low density deme fell well outside the other clusters. PERMANOVA calculations were done on both matrices (R v. 2.11.1, adonis(), vegan package; Oksanen et al. 2011) to determine if there was a significant difference due to deme.

T-tests were performed on individual characters, using unequal variances, to determine the characters driving overall differences in morphology (R. v. 2.11.1; R Development Core Team). P-values were considered after a table-wide Sequential Bonferroni correction (Rice 1989).

3.4 Behavioral Analyses

I collected swimming behavior data to compare to the data from Dawson & Hamner (2003) on the high density deme. Behavioral data on the low density deme were collected *in situ* following the methods of Dawson & Hamner (2003) for pulse rate, swimming speed, and the frequency and magnitude of turning. When possible, all measurements were taken on the same individual. In addition, bell diameter was measured *in situ* to the nearest half centimeter after all behavioral data were recorded. Medusae were selected haphazardly by choosing the first medusa seen by a diver after finning forward several kicks; for the most part, the population was of sufficiently low density that divers only saw one medusae at a time. To prevent repeat measurements on an individual, medusae were corralled temporarily in bags after being measured and not released after the end of measurements; medusae which were measured on different days were verified as new specimens by comparing photographs of distinguishing external morphological features and examining individuals for prior tissue sampling. A population census of the lake on the last day of measurements indicated I performed a thorough sampling of the medusae present. Temperature can affect pulse rate of *Mastigias* medusae (Dawson & Hamner 2003); temperatures across the top 3 m where *Mastigias* in the low density deme were measured was 31°C, within 1°C of the measurements from Dawson & Hamner (2003).

To control for effects of size, the size distribution of the low and high density demes were compared using an ANOVA for each measurement (different individuals were sampled for each behavioral measurement in the high density data) (Appendix II). If they were significantly different, further analyses used a generalized linear model which first accounted for size before the behavior in question. Pulse rates were related to bell diameter, each $\log_{10}$ transformed, and the slopes compared between demes with an

ANCOVA. Swimming speed had a slope statistically indistinguishable from zero when related to bell diameter, so an ANOVA was used to compare between demes. The magnitude of turning was calculated as the absolute difference between sequential bearings made *in situ* by medusae and the frequency calculated as any non-zero difference; they were compared between demes using an ANOVA. All analyses herein were performed in R (v. 2.11.1; R Development Core Team).

4 Results

4.1 Genetic Analyses

Haplotype networks for COI, COIII and the concatenated loci indicate similar haplotype composition in the high and low density populations (Figure 2, Appendix III). Individuals from cove locations from Swift et al. (2016) are shown for reference; the number per location for cove locations is a maximum of 15 (Swift et al. 2016). Both high and low density demes' haplotype composition clustered together, with a similar number of haplotypes in each network (COI: n_{high}=2, n_{low}=3; COIII: n_{high}=4, n_{low}=4; Concatenated: n_{high}=5, n_{low}=6). The low density deme only has two haplotypes unique from the high density deme in the concatenated network, comprised of 5 of the 38 individuals. The Φ_{ST} analysis indicates the overall pairwise difference between high and low density demes in the concatenated dataset is 0.03, which is not significantly different ($p = 0.095$). Φ_{ST} values between the NLK low density deme and cove locations, ranging between 0.48-0.71, indicate the low density deme is genetically significantly different from the coves ($p < 0.001$).

4.2 Morphological Analyses

The overall analyses of morphology indicate the morphology of medusae from high and low density demes are significantly different. The nMDS analysis (Figure 3) was a relatively good, reliable representation of the data (3 axis plot, stress = 0.07) and shows a wider spread of specimens through morphological space in the low density deme. PERMANOVA results indicate a significant difference due to density ($p < 0.001$).

The PCA analyses supported the nMDS results, with significant clustering by deme in each ($p < 0.001$, PERMANOVA). The PCA analysis with all variable characters included shows highly separated clustering by density level (Figure 4a). The two axes compose 0.56 and 0.25 of the proportion of variance explained. The second PCA analysis, which excluded three pigmentation characters found only in two low density deme individuals, shows similar results in that there is separation between the demes (Figure 4b). The two axes represent 0.78 and 0.09 of the proportion of variance explained. Both PCAs show two, very small juvenile (bell diameters = 15 and 22 mm), low density specimens which cluster with the high density individuals.

Comparisons of the individual characters reveal the specific morphological differences between high and low density deme individuals in 24 of 45 characters.

Individuals collected at low density were significantly larger than even the maximum size seen in the lake during high density (Figure 5, Table 2). The low density deme was significantly larger for both mean (high = 65.87 mm, low = 139.6 mm, $p < 0.001$) and mean maximum bell diameters ($p < 0.001$). Low density specimens were often pigmented, from exumbrellar spots and occasionally in their canals and terminal clubs, whereas pigmentation was absent from high density deme specimens ($p < 0.001$). The low density individuals also had more and longer terminal clubs which were more often tricorn in shape, rather than round ($p < 0.001$). Additionally, intermediate filaments on the oral arms and oral disc were more abundant in the low density specimens ($p < 0.001$). While these filaments often are doorknob-shaped, low density individuals also had many intermediate filaments which were long and shaped like terminal clubs (Table 2, Figure 1). Internally, low density specimens had significantly thinner bells and oral discs, and higher canal complexity ($p < 0.001$, Table 2).

4.3 Behavioral Analyses

The mean water column temperature where *Mastigias* medusae were measured between the two demes was within 1°C and unlikely to have caused a difference in pulse rate. The size distributions of the individuals sampled in the high density population for each metric of swimming behavior differed significantly from the individuals on whom data was collected in the low density population ($p < 0.001$, each comparison). Due to these differences, size was considered first in all statistical tests between the populations.

Pulse rate was significantly different between populations after accounting for size ($p < 0.005$), with bell diameter and population representing 0.875 of the variation (Table 3, Figure 6a). Swimming speed was also significantly different between populations after accounting for size ($p < 0.001$), although size and population only represented 0.429 of the variation in swimming speed (Table 3, Figure 6b). The mean degree magnitude of turns was 13° less in low density than high density individuals (Table 3). Additionally, the curve of degree magnitudes was shifted towards smaller degree turns in the low density population, shown by the histogram of all turn magnitudes (Figure 6c), and the higher fraction of turns in the low density population (Table 3). However, once variation due to size was accounted for in metrics of turning and significance was adjusted based on sequential Bonferroni corrections, no metric of turning was significantly different between high and low density populations, although the turn magnitude and percentage of turns ≤ 20° both were close to the cut-off (Table 3).

5 Discussion

In 2011, the population of ~4-5 million medusae (~5-7 medusae m^{-3}) in NLK declined to zero within a few months and medusae were absent until the following year, when low density of medusae were present (<0.05 medusae m^{-3}). Comparisons between the low and high density demes found significant differences in (a) morphology, and (b) swimming behavior, but not (c) genetic composition. Significant morphological

differences manifested mainly as larger, pigmented animals with longer and tricorn-shaped terminal clubs, greater number of intermediate filaments, thinner mesoglea and greater internal canal complexity during lower density. Differences were also found, even after correcting for medusa size, in slower pulse rates and higher swimming speeds of medusae and, to a lesser extent, lower turning magnitude, although not the frequency of turns. Hypothesized mechanisms underlying changes in morphology and behavior following the environmental perturbation are 1) recolonization of the lake, 2) a rare genotype that is differentially represented under these post-crash conditions, or 3) plasticity. The genetic signature in mtDNA in the low density deme is indistinguishable from the high density deme in NLK, but statistically distinct from any of the cove locations.

5.1 Sources of variation

Genetic analyses, which indicate the low density deme's significant difference from the cove and similarly to the high density NLK deme, largely excludes the possibility that the behaviorally and morphologically distinct medusae had origins outside of NLK, negating the first hypothesis. The behavioral and morphological variation observed, therefore, is a manifestation of variation within the population that is endemic to NLK.

Although the Φ_{ST} values indicate that the low and high density specimens are from the same gene pool, the small fraction of the genome sequenced makes it impossible to distinguish between the remaining two hypotheses. Additionally, each hypothesis can be supported by other studies, making it difficult to favor one over the other. Under stressful environmental conditions (akin to those present in NLK upon medusae recovery), *Symbiodinium* density in polyps decreases, as does the proportion of polyps strobilating (Dawson et al. 2001). It is possible that under these conditions, differential strobilation occurred which favored a rare polyp genotype that also produces the variation in morphology and behavior in the medusae. There is also prior evidence of phenotypic plasticity in *Mastigias*, as aquarium-raised *Mastigias* medusae display ecophenotypic variation, although not enough to overwhelm site-specific morphologies (Dawson 2000, 2005a). Ontogenetically, phenotypic differences accrue with growth, and small medusae from different lakes (Dawson 2005a) or demes (Figure 4) are more similar in morphology than larger. Ecophenotypic variation between demes in NLK could thus possibly be plastic responses during the growth of the medusae, not necessarily a different genotype. Distinguishing between the two hypotheses would need both expanded genomic analyses and raising genetically similar individuals under the post-perturbation conditions until they were large enough to possibly display morphological differences.

In either scenario, there is an opportunity to study possible parallel patterns of ecophenotypic variation. A similar environmental perturbation, consisting of a stratified water layer of increased temperature and salinity and drastic *Mastigias* medusae population crash followed by medusae morphological and behavioral variation upon recovery, has occurred in at least one other lake in Palau (Ongeim'l Tketau, Dawson et al. 2001, Dawson 2005a). Thus, the shared ecophenotypic response could be due to parallel

or convergent patterns or sources of variation. Either scenario presents a unique opportunity to study ecophenotypic responses to perturbations in a marine organism.

Whether characteristics in the post-crash phenotype are a result of warmer temperature, low density, or other environmental changes, acting alone or in concert, remains unclear. It is possible the larger maximum size of post-perturbation medusae is due to low density, and thus lack of competition for zooplankton prey from several million other medusae, assuming a similar planktonic community. Temperature could also have an effect on post-crash phenotype. Medusae observed *in situ* in the low density deme were at 3m maximum depth, regardless of the degree of cloud overcast, staying above the abrupt thermocline ~5m depth. The mode of distribution with depth of NLK *Mastigias* pre-perturbation was 8m when it was sunny and 2m when it was cloudy, due to short-term behavioral adjustments (Dawson & Hamner 2003). This adjustment has been hypothesized to be behavioral photoprotection (Dawson & Hamner 2003) in medusae that are without blue pigmentation, which has been hypothesized to be photoprotective (Blanquet & Phelan 1987). *Mastigias* from the low density deme do have increased pigmentation, but mostly from spots and flecks, not the blue bell; whether these pigmentations also have photoprotective qualities is unknown, but the behavioral responses to cloud cover do seem affected by the thermocline. Without more specific experimental evidence on the causes and consequences of a) character changes in alternative phenotypes, b) characters that are specifically triggered by the environment, and c) characters that are swept along due to character complexes or gene linkage, any potential adaptive benefit is unclear.

5.2 Ecological implications of morphological and behavioral variation

Morphology, swimming and prey capture are mechanistically interrelated in medusozoans (Costello & Colin 1995, D'Ambra et al. 2001). Characters such as size, pulse force, pulse rate and swimming speed affect medusae swimming (Costello & Collin 1995, D'Ambra et al. 2001). In *Mastigias,* pulse rate is strongly related to bell diameter (Dawson & Hamner 2003), such that swimming speed is approximately normally distributed. Even so, larger medusae have a higher clearance rate at the same swimming speed as smaller medusae due to the larger volume of the entrained flow (Arai 1997). In the low density deme, pulse rate and swimming speed are elevated and the medusae are larger, suggesting a higher clearance rate in the low density medusae than the high density medusae. In fact, the mean pulse rate and swimming speed of low-density medusae from NLK are similar to medusae in the shallow holomictic lake OLO and cove RCA (Dawson & Hamner 2003). However, the turning behavior did not significantly change, although the mean magnitude of turns did decrease. Prior hypotheses regarding the increased turning in lake medusae suggested that larger size and longer terminal clubs in cove medusae could increase swimming efficiency in straight lines (Dawson & Hamner 2003, Dawson 2005a). This pattern did not emerge in the low density medusae, which may indicate that a suite of features control swimming efficiency, such as paired long terminal clubs and large size with a robust, thick bell or the increased ratio of terminal club length to bell diameter, both of which are characteristic of cove medusae, but not the low density deme. Or it could be because the low-density ecotype terminal

clubs are still quite different from the lagoon types. Alternatively, turning could be an evolved behavior, as other horizontal migration behaviors have evolved separately in lakes (Dawson & Hamner 2003). Thus, ecophenotypic variation could have subsequent differential effects on plankton communities. To better understand the relationship between ecophenotypic variation and community ecology, further investigation of the link between morphological characteristics and swimming behavior is needed.

The kinds of variation recorded in pulse rate, swimming speed and bell diameter affect the marginal velocity that determines the prey entrained in flow into capture surfaces in other rhizostomes and scyphomedusae (Costello & Colin 1994, Ford et al. 1997, D'Ambra et al. 2001). Although *Mastigias* harbors photosynthetic *Symbiodinium,* the daily nutrient requirements of *Mastigias* medusae in the lakes are not met by symbiosis alone (McCloskey et al. 1994), although the diet of *Mastigias* medusae is unknown. Under pre-perturbation conditions, *Bestiolina inermis* and bivalve larvae comprise a mean proportion of ~0.75-0.81 of the plankton community in NLK and *Oithona oculata,* copepod nauplii and fish eggs comprise the next 0.17-0.23 (1996-97: Martin 1999, 2003-2008: Appendix IV). Evidence from other scyphozoans indicates that plankton with calcareous shells are ingested, but expelled alive (Purcell et al. 1991). Subsequently, these data indicate that, at least, the largest fraction of prey available to *Mastigias* medusae are actively escaping copepods.

Copepod species have varying hydrodynamic sensitivities that trigger escape responses from predators, so they are captured when predators have higher flow velocities than the velocity of the escape response or if turbulence masks predator's velocities from the copepods (Waggett & Buskey 2007, Buskey et al. 2002, Gilbert & Buskey 2005). Ecophenotypic variation in *Mastigias* seems to correspond to these two scenarios: medusae in low density environments have slower pulse rates and larger maximum size which generally act to increase marginal flow velocity (D'Ambra et al. 2001) to entrain actively escaping prey, while medusae in high density environments are masked by turbulence produce from millions of medusae swimming in different directions. Without knowing the flow velocities of *Mastigias,* it is impossible to know for sure if the flow velocity is greater than the escape velocity of the copepods in NLK.

5.3 Implications for environmental variability research on medusozoans and marine systems

Perturbations can have disproportionately large impacts relative to their duration, and may set up different reactions and dynamics than sustained climatic trends (Jentsch et al. 2007, Brierley & Kingsford 2009). Perturbation induced responses are evident across scales, with ecological and evolutionary ramifications for populations and communities. Evolutionarily, perturbations may shift selection landscapes for populations and result in differential intraspecific variation through phenotypic plasticity, differential expression of genotypes, or other microevolutionary processes. The prevalence and importance of these sources of intraspecific variation as responses to perturbations is not known for marine systems. Variable responses could completely change the otherwise expected response to perturbations or trends, especially if it can facilitate adaptive evolution (Price et al. 2003, West-Eberhard 2003, Pfennig et al. 2010). In light of increasing perturbations, we need to

explore how perturbations and intraspecific variation shape reactions and dynamics from populations to ecosystems, especially in marine systems.

Studying how perturbations and intraspecific variation shape dynamics will benefit from delineating natural populations in marine systems. In scyphozoans, a clade for which there is heightened interest in responses to climatic variation, there has been limited discussion of the role of intra-specific spatio-temporal variation. Most studies which try to correlate fluctuations in medusa abundance and climate indices focus on large-scale trends, which may obscure the local responses and signals to which populations respond (Dawson et al. 2015). For example, although OTM and NLK are only ~23 km apart, when the population of OTM crashed in response to warm temperatures from the 1997/8 ENSO event (Dawson et al. 2001, Martin et al. 2006), the population in NLK was relatively stable (Dawson et al. 2001). Conversely, when the population of NLK crashed in 2011, the population of OTM medusae remained (Dawson, unpublished data). The variable and differential responses of *Mastigias* populations to perturbations illustrate the need for study at the population and local level to understand the responses of scyphozoans to climate variability. The potential for some marine systems to function as 'islands' (Dawson 2015, Dawson et al. 2016), and the use of these 'natural laboratories' to answer evolutionary questions (Hachich et al. 2015, Swift et al. 2016) opens new avenues for addressing plasticity and perturbations in the marine realm.

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7 Tables & Figures

Figure 1. Examples of representative morphologies of *Mastigias papua* medusae in NLK under high density (*top row*) and low density (*bottom row*) conditions. Medusae not to scale; sizes for medusae in top row range from ~8-10 cm bell diameter (bd). Medusae in the bottom row range from 11-20 cm bd. The high density spotted form (*top right*) occurs at frequency ~2% of the population; the unspotted forms occur at frequency ~98% (*top left, top center*) (Dawson 2005b). The unspotted form in the low density population occurred at ~9% frequency ($n = 23$) and the spotted form at ~91% frequency. Also note the differences in length and shape of the terminal clubs and the presence and abundance of intermediate filaments and terminal-club-like appendages on the oral arms of the low density medusae that are generally absent or at low frequency on the high density medusae. Photographs of high density medusae by MN Dawson (*top center*) and LE Martin (*top left, top right*).

Table 1. Number of specimens used for genetic and morphological analyses, excluding *in situ* measurements for size. Data from previous work are indicated with superscripts; all other data were analyzed for this project. The genetic analyses for August 2012 – August 2013 are from individuals that are included in the morphological analyses for that time period. All other genetic and morphological specimens are not from the same individuals. Total morphological specimens are the maximum number that could have been used in any of the other three morphological analyses.

	Genetic Analyses	Morphological Analyses			
		Total Specimens	Morphometrics		Color Traits
			Unpreserved	Preserved	
Pre-Perturbation					
1996 – 2001	15[ab]	19[a]	19[a]	n.a.	19[a]
1997 – 2006	23	n.a.	n.a.	n.a.	n.a.
Post-Perturbation					
August 2012 – August 2013	38	28	n.a.	28	n.a.
June – July 2015	n.a.	23	4	8	23

[a] from Dawson (2005a) [b] from Swift et al. (2016)

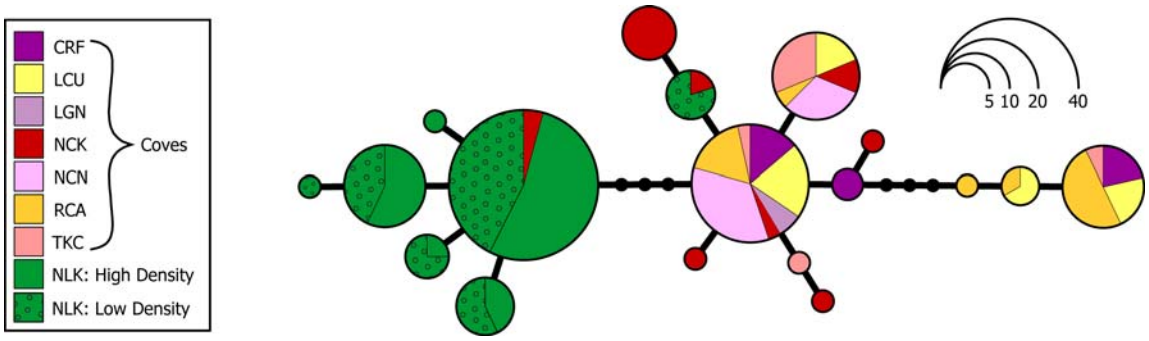


Figure 2. Concatenated COI and COIII haplotype network showing the high and low density populations from NLK. Each branch corresponds to one nucleotide difference; unsampled haplotypes are indicated by a black dot. Circle area is proportional to the number of individuals with that haplotype in reference to the scale. Cove locations (warm colors) are from Swift et al. 2016 and Dawson & Hamner 2005 and provided for reference to previously published networks; the maximum number of individuals for any cove location is 15. The NLK populations are dark green, with the circles overlaid on the low density population. NLK samples n = 38 for each deme; the high and low density populations in NLK are not significantly different in terms of haplotypes.

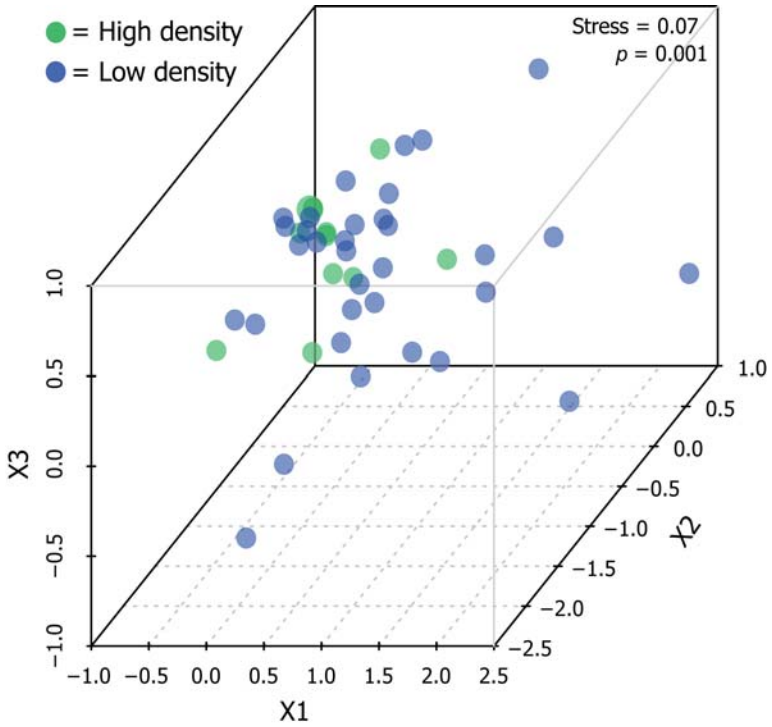


Figure 3. Morphological similarity of high (green) and low (blue) density *Mastigias* medusae morphology plotted in three dimensions by non-metric multi-dimensional scaling (nMDS) of quantitative characters. Lower stress values represent better representation of the data by the plot; values under 0.1 are considered a good fit. PERMANOVA tests indicate that density is a significant predictor of overall morphological differences between demes ($p = 0.001$).

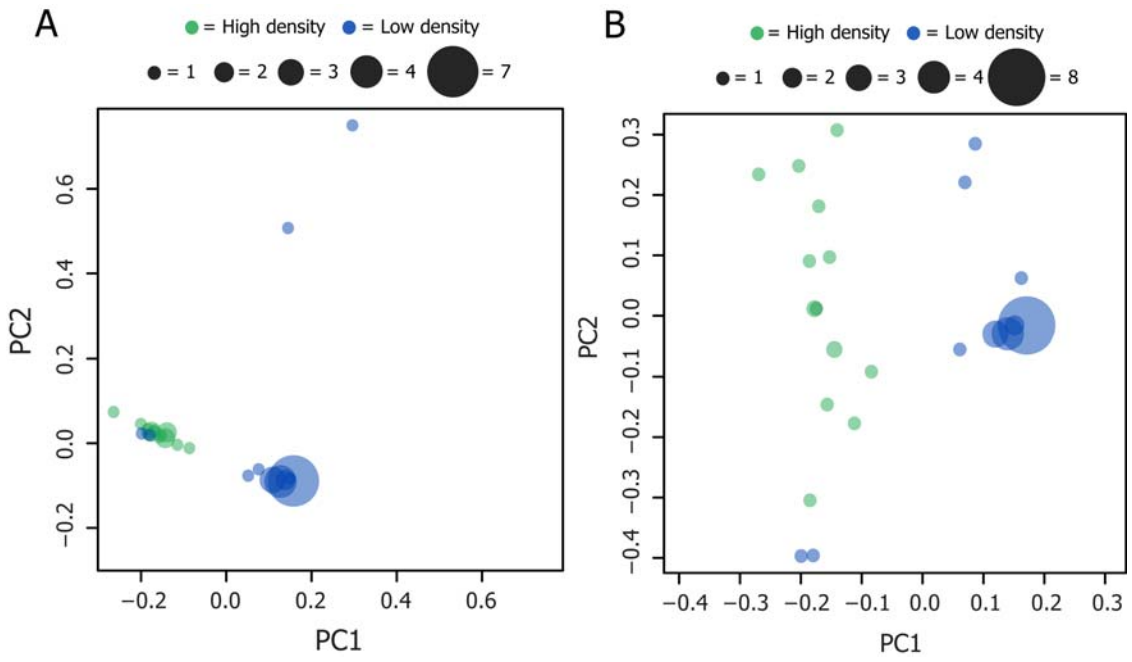


Figure 4. Morphological similarity of *Mastigias* medusae in high (green) and low (blue) density from categorical principal component analysis (PCA) of qualitative characters. The size of the circle represents the number of individuals that shared the same coordinate. **A** PCA analysis including all individuals. The two individuals separate from the cluster segregate based on three pigmentation characters, the presence of blue pigment on the terminal clubs and in the canals and white pigmentation on the oral arm fringe, which comprise the majority of the variation on PC2. **B** PCA analysis with the two blue individuals removed, to facilitate differentiation along two axes of the main cluster. PERMANOVA tests on both sets indicated a significant difference due to density ($p = 0.001$).

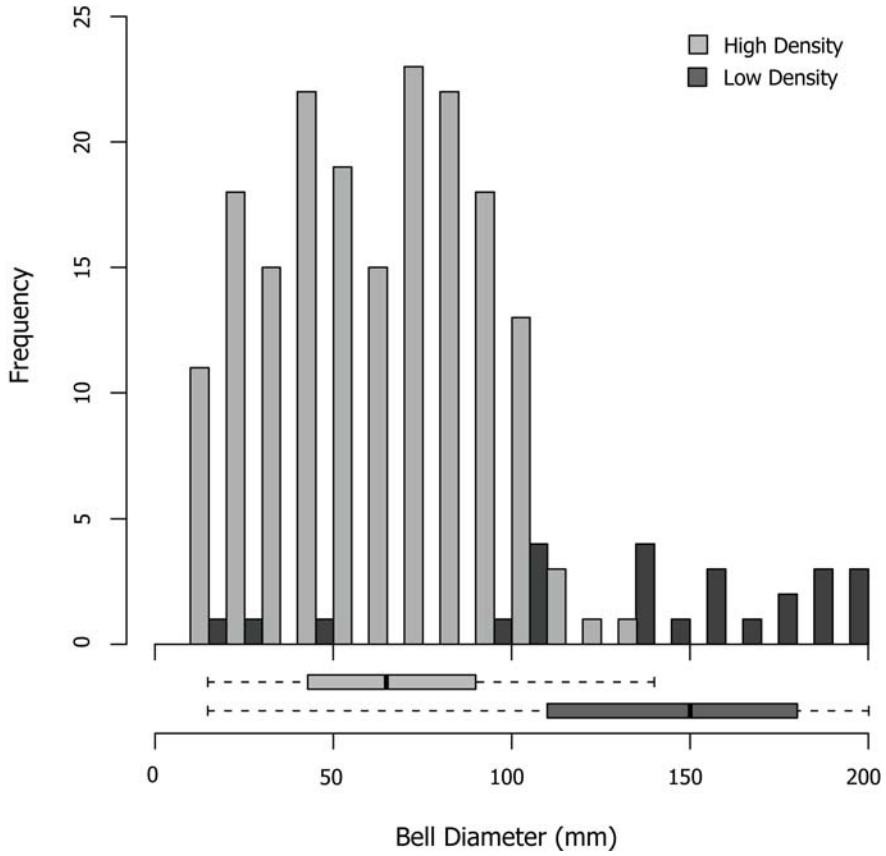


Figure 5. Bell diameter histogram and box plots for high and low density *Mastigias* medusae populations. Boxplots below the histogram illustrate the mean and spread of the bell diameters for each population. High density data came from aggregating the swimming behavior data to provide a representative population sample from November 1996- May 1997 (Dawson & Hamner 2003); low density data was from *in situ* measurements in July 2015. Although the number sampled in 2015 is lower, it represented a complete sampling of the population present at that time.

Table 2. Summary of all continuous and categorical features measured in *Mastigias* high and low density populations. Type indicates if the feature was continuous (Cont.) or categorical (Cat.) and, if the latter, the states possible. State codes are as indicated in Dawson (2005a). Features which were significantly correlated with bell diameter (bd), and thus a ratio of the feature / bd was used in analyses and displayed here is indicated in the Ratio column. Features for which there was a significant difference between high and low density populations, after table-wide Sequential Bonferroni correction (Rice 1989) are indicated with asterisks.

			Cat: Proportion; Cont: Mean (SE)		
Feature	Type	Ratio	High	Low	*p*
Exumbrellar blue pigmentation	Cat.: y/n	-	1.0 n	1.0 n	
Canal fleck abundance	Cat.: n, 1, 2	-	1.0 n	0.09 n, 0.39 1, 0.52 2	*
Canal blue pigmentation	Cat.: y/n	-	1.0 n	0.91 n, 0.09 y	
Oral arm pigmentation on fringe	Cat.: w, y, b	-	0.0 w	0.04 w	*
Terminal club pigmentation	Cat.: w, b, y	-	NA w, 0.0 b	0.74 w, 0.09 b	
Exumbrellar spot abundance	Cat.: n, 1, 2, 3	-	1.0 n	0.09 n, 0.04 1, 0.26 2, 0.61 3	*
Exumbrellar spot color	Cat.: w, y, g	-	NA	1.00 w	NA
Terminal club shape – minimum	Cat.: 0, 1, 2, 3	-	0.68 0, 0.32 2	0.09 0, 0.04 2, 0.87 3	*
Terminal club shape – maximum	Cat.: 0, 1, 2, 3	-	0.11 0, 0.79 2, 0.11 3	0.09 0, 0.91 3	*
Oral arm intermediate filament abundance	Cat. : n, 1, 2, 3	-	0.63 n, 0.37 1	0.09 n, 0.04 2, 0.87 3	*
min. shape	Cat.: 0, 1, 2, 3	-	NA	1.0 0	NA
max. shape	Cat.: 0, 1, 2, 3	-	NA	0.1 0, 0.9 3	NA
Oral disc intermediate filament abundance	Cat.: n, 1, 2, 3	-	1.0 n	0.13 n, 0.87 3	*
Bell diameter[†]	Cont.	-	65.87 (2.10)	139.6 (10.37)	*
Ring canal diameter	Cont.	y	0.82 (0.007)	0.83 (0.005)	
Oral arm unwinged length	Cont.	y	0.11 (0.007)	0.08 (0.005)	*
Oral arm winged length	Cont.	y	0.17 (0.010)	0.27 (0.007)	*
Terminal club number	Cat.: 0-8	-	4.69 (0.59)	7.53 (0.14)	*
Terminal club length	Cont.	-	8.44 (0.93)	30.50 (3.74)	*
Oral pillar length	Cont.	y	0.04 (0.003)	0.04 (0.003)	
Oral pillar width	Cont.	y	0.07 (0.003)	0.08 (0.002)	
Oral pillar thickness	Cont.	y	0.02 (0.001)	0.03 (0.003)	
Subgenital ostia width	Cont.	y	0.31 (0.008)	0.29 (0.003)	
Oral disc diameter	Cont.	y	0.41 (0.01)	0.42 (0.007)	
Oral disc thickness at edge	Cont.	-	2.58 (0.25)	1.27 (0.11)	*
Oral disc thickness 2/3	Cont.	-	6.66 (0.53)	2.92 (0.32)	*
Oral disc thickness 1/3	Cont.	-	1.79 (0.18)	1.06 (0.04)	*
Oral disc thickness at center	Cont.	-	1.21 (0.25)	1.07 (0.04)	
Lappet number	Cont.	y	0.17 (0.010)	0.12 (0.006)	*

Gastrovascular cavity diameter interradial	Cont.	y	0.54 (0.008)	0.54 (0.006)	
perradial	Cont.	y	0.29 (0.01)	0.27 (0.01)	
Bell thickness 4/5	Cont.	y	0.03 (0.002)	0.02 (0.002)	*
Bell thickness 3/5	Cont.	-	5.53 (0.43)	3.03 (0.50)	*
Bell thickness 2/5	Cont.	y	0.08 (0.001)	0.04 (0.004)	*
Bell thickness 1/5	Cont.	-	4.97 (0.28)	3.29 (0.68)	
Bell thickness center	Cont.	-	4.37 (0.26)	3.24 (0.70)	
Perradial canal origins	Cont.	-	1 (0)	1 (0)	
Interradial canal origins	Cont.	-	1 (0)	1.01 (0.01)	
Adradial canal origins	Cont.	-	7.5 (0.31)	11.33 (032)	*
Perradial canal anastomoses	Cont.	-	0.31 (0.08)	0.54 (0.10)	
Interradial canal anastomoses	Cont.	-	0.17 (0.06)	0.35 (0.07)	
Adradial canal anastomoses	Cont.	-	10.95 (0.66)	40.13 (4.41)	*
Sinuses from the gastrovascular cavity	Cont.	-	0.29 (0.07)	0.89 (0.14)	*
perradial canal	Cont.	-	0.06 (0.04)	0.12 (0.05)	
interradial canal	Cont.	-	0.04 (0.03)	0.11 (0.03)	
adradial canals	Cont.	-	3.54 (0.76)	9.47 (0.81)	*
ring canal	Cont.	-	0.34 (0.12)	1.48 (0.18)	*
Double sinuses	Cont.	-	0.28 (0.13)	0.47 (0.10)	

† Comparison of full populations from *in situ* data, not specimens measured for morphology, due to the targeted size classes for morphometric analysis of the high density population.

Table 3. Summary of swimming behavior between high and low density *Mastigias* medusae. Temperatures at which high and low density population medusae were measured are within 1°C. Significant difference, after table-wide sequential Bonferroni correction, of a swimming metric between the populations is indicated with an asterisk.

Swimming Behavior Metric	Mean (SE)		p-value[a]	*p*
	High	Low		
Pulse Rate[b] (#/s)	2.19 (0.10)	1.55 (0.10)	0.0046	*
Swimming Speed (cm/s)	4.38 (0.08)	5.72 (0.42)	4.86×10^{-5}	*
Percentage of turns = 0	16 (1.82)	15 (2.25)	0.439	
Percentage of turns ≤ 20°	44 (3.75)	70 (4.19)	0.046	
Turn Magnitude	35 (1.39)	22 (1.38)	0.062	

[a] after accounting for size in the linear model. [b] means are for untransformed pulse rate, while *p*-values are from the formula lnPR~lnBD+Population

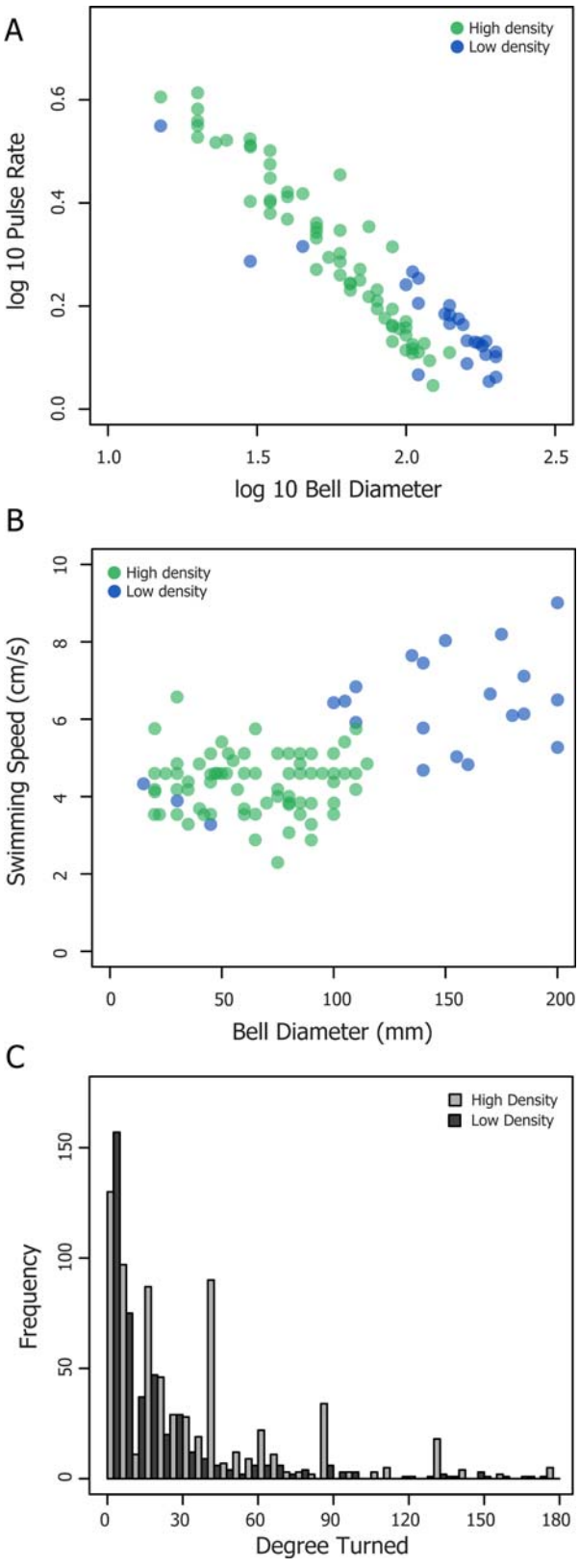
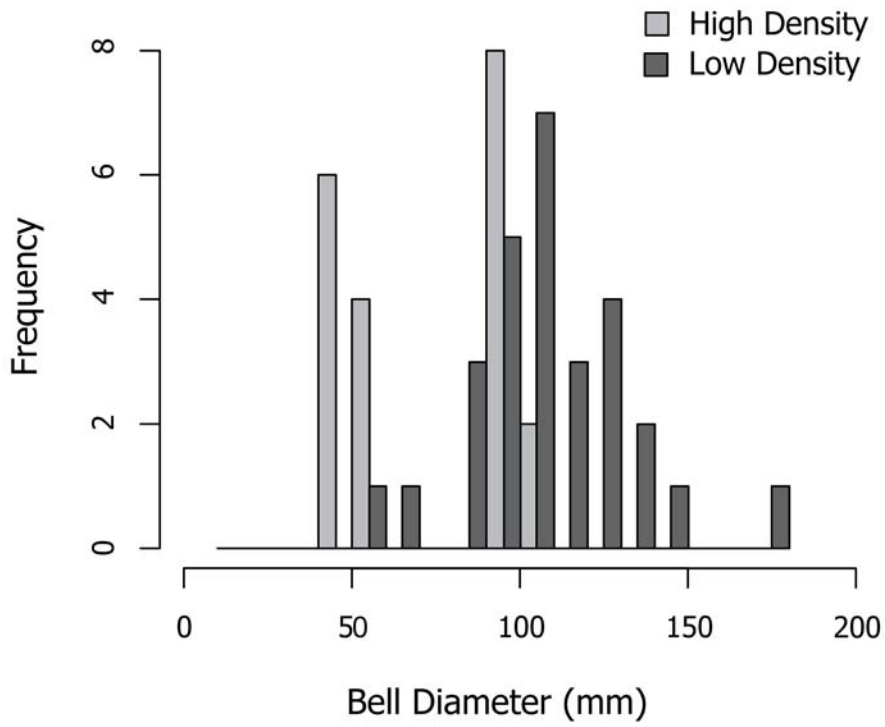
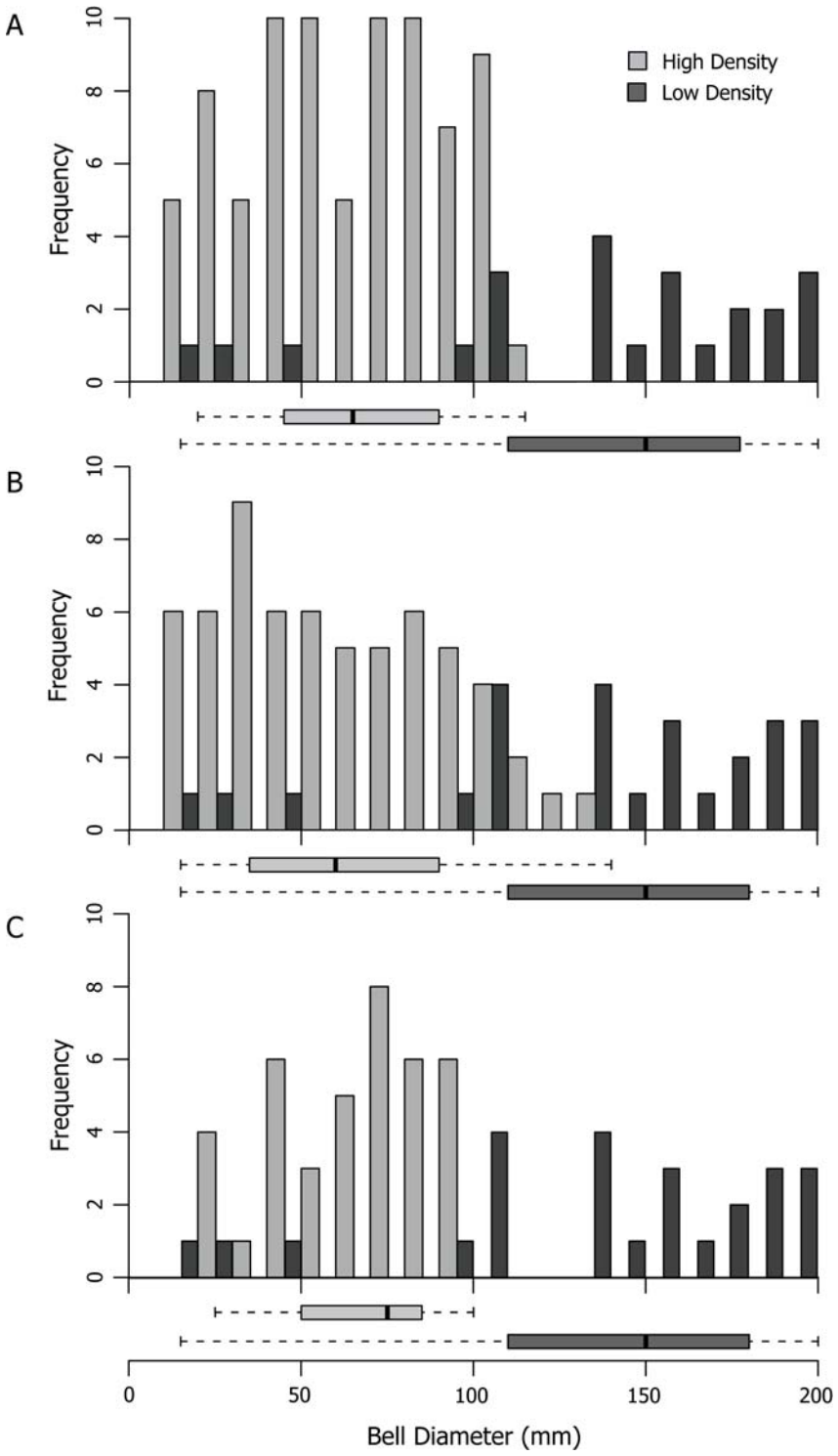

A
High density
Low density
log 10 Pulse Rate
0.0
0.2
0.4
0.6
1.0
1.5
2.0
2.5
log 10 Bell Diameter
B
High density
Low density
Swimming Speed (cm/s)
0
2
4
6
8
10
0
50
100
150
200
Bell Diameter (mm)
C
High Density
Low Density
Frequency
0
50
100
150
0
30
60
90
120
150
180
Degree Turned

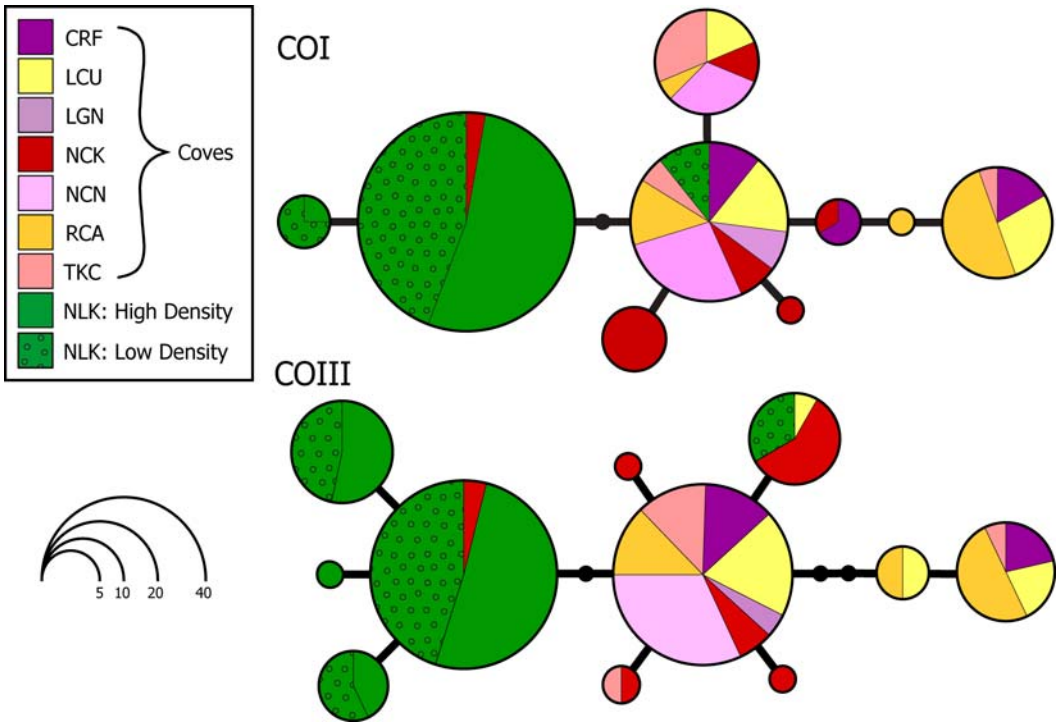
Figure 6. Swimming behavior of high and low density *Mastigias* medusae. Temperatures at which high and low density population medusae were measured are within 1°C. **A** Log_{10} transformed pulse rate (pulses s^{-1}) against log_{10} transformed bell diameter (bd) of high (green) and low (blue) density *Mastigias* medusae populations. Pulse rate was significantly different between high and low density populations after accounting for bd ($p = 0.0046$). **B** Swimming speed (cm s^{-1}) plotted against bd of high (green) and low (blue) density *Mastigias* medusae populations. Swimming speed was significantly different between the populations after accounting for bd ($p < 0.0001$). **C** Turning degree magnitude frequencies for high (light grey) and low (dark grey) *Mastigias* medusae populations. Although not significantly different after a table-wide Sequential Bonferroni correction on all swimming metrics, there is a shift in the low density medusa population towards lower degree magnitude turns.



Appendix I. Frequency of bell diameters for the high (light grey) and low (dark grey) density *Mastigias* medusae populations for specimens which were used in morphological analyses.



Appendix II. Frequency of bell diameters for the high (light grey) and low (dark grey) density *Mastigias* medusae population used in swimming behavior analyses of a) swimming speed, b) pulse rate and c) turning. Boxplots below each histogram illustrate the mean for each population.



Appendix III. Haplotype networks of the individual loci, COI and COIII. Each branch corresponds to one nucleotide difference; unsampled haplotypes are indicated by a black dot. Circle area is proportional to the number of individuals with that haplotype in reference to the scale. Cove locations (warm colors) are from Swift et al. 2016 and Dawson & Hamner 2005 and provided for reference to previously published networks; the maximum number of individuals for any cove location is 15. The NLK populations are dark green, with the circles overlaid on the low density population. NLK populations n = 38 each.

Appendix IV. Proportion of total microzooplankton present in Ngermeuangel Lake (NLK). The means from four subsamples per specimen (date-basin) were calculated prior to determining the proportion each category comprised of the total zooplankton assemblage. Data from Martin 1999: pg 69.

Source	Date	Basin	*Oithona oculata*	*Bestiolina inermis*	Copepod nauplii	Bivalve larvae	Veliger	Egg	Nereid larvae	Sea Star larvae
hfs										
	Mar 2003	S	0.046	0.655	0.007	0.283	0.005	0005	0.000	0.000
	May 2003	N	0.166	0.293	0.006	0.517	0.004	0.014	0.000	0.000
	June 2003	N	0.147	0.452	0.071	0.239	0.019	0.071	0.000	0.000
	July 2003	N	0.108	0.585	0.077	0.171	0.016	0.043	0.000	0.000
	Aug 2003	N	0.154	0.546	0.090	0.153	0.004	0.053	0.000	0.000
	Sept 2003	S	0.057	0.538	0.060	0.285	0.017	0.042	0.000	0.000
	Jan 2004	N	0.007	0.593	0.087	0.285	0.024	0.002	0.002	0.000
	May 2006	N	0.022	0.740	0.070	0.138	0.014	0.016	0.000	0.000
	June 2007	N	0.087	0.442	0.073	0.320	0.058	0.019	0.000	0.000
	June 2008	N	0.030	0.663	0.067	0.211	0.020	0.001	0.000	0.007
	MEAN		**0.082**	**0.551**	**0.061**	**0.260**	**0.018**	**0.027**	**0.000**	**0.001**
Martin 1999										
	May 1996		0.030	0.500	0.204	0.280	0.010	NA	NA	NA
	May 1997		0.000	0.450	0.223	0.280	0.030	NA	NA	NA
	MEAN		**0.015**	**0.475**	**0.214**	**0.280**	**0.020**	**NA**	**NA**	**NA**

CHAPTER 4:

ENVIRONMENTAL CAUSES AND SYNECOLOGICAL CONSEQUENCES OF DRAMATIC FLUCTUATIONS IN *MASTIGIAS* (SCYPHOZOA, RHIZOSTOMEAE) POPULATION SIZE

1 Abstract

The factors driving jellyfish population dynamics are poorly understood, due partly to a lack of understanding of the geographic scale of populations. Populations bloom, so analyses at scales larger than populations could decouple the causal influences from the observed effect, obscuring the factors that cause a population to bloom. Studies in marine lakes resolve this issue by utilizing discrete, isolated populations. Within marine lakes in Palau, the abundance of *Mastigias papua* medusae, which form the top of a pelagic planktivorous trophic chain, has fluctuated fifty-fold interannually between March 1999 and December 2008. I analyzed changes in the population sizes of medusae in 'Jellyfish Lake,' their planktonic prey, and environment for 10 years following the major El Niño-La Niña event of 1997/8. There was a rapid demographic expansion and overall mean population size larger than pre-1997 population size estimates with high variability for the first seven years, until returning to lower mean abundance and more stable population size approximately equivalent to the pre-1997 population size. *Mastigias* population fluctuations were strongly associated with mean mixolimnion temperature (0.67 of the variance explained) and salinity, dissolved oxygen concentration and pH to a lesser extent. Similar patterns of association with environmental variables are displayed across all *Mastigias* size classes. However, the pattern of association varies; temperature has little effect on *Mastigias* medusae density until a critical temperature threshold where there is a steep, strong negative effect, while medusae association with salinity displayed a continuous gradual negative association. *Mastigias* medusae had negative, moderately strong associations with zooplankton (0.26-0.39 of the variance explained), indicative of top-down predator effects. This sustained study of a single population's dynamics over multiple years suggests there may be many factors of approximately equivalent maximum effect influencing jellyfish bloom dynamics, with the relative importance of these factors varying through time.

2 Introduction

The factors driving scyphozoan population dynamics are poorly understood (West et al. 2009, Condon et al. 2013), especially in tropical systems. The importance of scyphozoans, as pelagic predators (Mills 1995, Behrends & Schneider 1995, Purcell & Arai 2001, Purcell 2005), commercially important fisheries (Omori & Nakano 2000), and successful invasive species (Holland et al. 2004), has led to interest in scyphozoan responses to climate variability (Purcell 2005, Richardson et al. 2009, Brotz et al. 2012,

Condon et al. 2013). Yet, most of the studies have been large-scale, although genetic differentiation (Abboud et al. in prep, Lee et al. 2013) and dynamics of populations occur on regional and local scales (Dawson et al. 2015). Since populations bloom (Graham et al. 2001), analyses at scales larger than populations could decouple the causal influences from the observed effect, obscuring the factors that cause a population to bloom (Dawson et al. 2015).

There is ample evidence of responses to contemporary climate variability at the species level, but little investigation of community or ecosystem level responses (reviewed in Walther et al. 2002, Montoya & Raffaelli 2010, Walther 2010). For scyphozoans, recent studies have suggested that climatic forcing dominate plankton-jellyfish trophic interactions and may determine the occurrence of jellyfish blooms (Condon et al. 2013). However, it remains unclear whether jellyfish exert top-down influences as an important pelagic predator (Verity & Smetacek 1996, Schneider & Behrends 1998, Stibor et al. 2004), if bottom-up factors influence jellyfish populations (Gibbons & Richardson 2009, Garcia-Comas et al. 2011) or if both can occur simultaneously (Pitt et al. 2007, West et al. 2009). Furthermore, how bottom-up and/or top-down control may vary as environmental conditions change has not yet been explored. Thus, in addition to a need for investigations of scyphozoan responses to climatic variability at the appropriate scale, it is critical we also investigate whether their entire pelagic planktonic community or their roles within it shift with environmental fluctuations.

The uncertainty, both in scyphozoan species and ecosystem responses, lies partly with the complex and open systems in which scyphozoans occur and partly with our nescience of the geographic scale of populations. Studies in marine lakes – small bodies of seawater entirely surrounded by land – resolve this issue by utilizing discrete, isolated populations. These simplified microcosms of the complex 'mainland' ocean provide tractable systems to study evolutionary, ecological and trophic dynamics, much as oceanic islands can for mainland systems (MacArthur & Wilson 1969, Dawson 2015, Dawson et al. 2016)

Mastigias papua is a rhizostome jellyfish that lives in coves and marine lakes throughout the Indo-West Pacific (Kramp 1961, Swift et al. 2016). Within the marine lakes in Palau, the abundance of *M. papua* medusae, which form the top of a pelagic trophic chain, has fluctuated fifty-fold interannually between March 1999 and December 2008. First publications on the marine lakes indicated the population of *M. papua* and environmental characteristics of the lake were relatively stable (Hamner et al. 1982, Hamner & Hamner 1998). The dramatic crash of the *M. papua* medusa population following a warming event from the El Niño-La Niña event of 1997/8, and its subsequent recovery (Dawson et al. 2001; Martin et al. 2006) dispelled this notion. Prior analysis indicated the *M. papua* population size was negatively correlated with mixolimnion temperature (Martin et al. 2006), possibly due to a decrease in strobilation of benthic polyps producing ephyrae (juvenile medusae) and reduced survivorship of those ephyrae (Dawson et al. 2001). The simplified food chain in the marine lake and the isolated population provides an opportunity rare in its clarity to study medusae responses to environmental fluctuations and trophic structure. I analyzed changes in the population densities of medusae in Ongeim'l Tketau (the famous 'Jellyfish Lake'), their planktonic

prey, and environment for 10 years following the major El Niño-La Niña event of 1997/8 to determine environmental and synecological causes or consequences of population fluctuations. Specifically, I sought to determine 1) what influences *Mastigias* population fluctuations, and 2) what consequences did medusae population fluctuations have for the rest of the pelagic plankton community.

3 Methods

3.1 Study system

Marine lakes occur throughout the Indo-West Pacific in karst landscapes (Dawson et al. 2009). Ongeim'l Tketau (OTM), Palau, is perhaps the most famous marine lake due to its popularity with tourists who wish to swim with millions of jellyfish (Colin 2009). Although the shortest distance overland from the lake to the ocean is only 150 m (Dawson & Hamner 2005), the lake is essentially isolated from the ocean, with limited hydrodynamic exchange with the ocean (Hamner & Hamner 1998) and genetic isolation of at least two species (Dawson & Hamner 2005, Gotoh et al. 2009). ^{14}C dating indicates that the lake formed after the Last Glacial Maximum, roughly 10,000 years ago (Smittenburg et al. 2011). OTM is situated along an east-west axis with two basins, the westward basin roughly 30 m deep and the eastward basin approximately 25 m deep, with a slightly narrower and shallower sill between the basins (Hamner & Hamner 1998). Water temperature generally ranges between 30°C and 32°C, declining slightly with depth (Hamner & Hauri 1981, Hamner & Hamner 1998, Dawson & Hamner 2003); salinity ranges between 24psu and 29psu, depending on recent rainfall, and increases slightly with depth (Hamner & Hauri 1981, Hamner & Hamner 1998, Dawson & Hamner 2003). Oxygen declines steadily in the lake until 12-15 m depth, below which is anoxic sulfide-rich water to the lake bottom (Hamner & Hauri 1981, Hamner & Hamner 1998, Dawson & Hamner 2003).

The lake is lined with mangroves, on which roots live a variety of benthic invertebrates; below the reach of the mangrove roots is mainly alga and occasional clusters of invertebrates, most notably a medusivorous anemone wherever hard substrate emerges from the otherwise fine silt basin. In the water column, the *Mastigias papua* jellyfish is the most visible organism, although there can be small schools of striped silverside fish, *Pranesus eendrachtensis*, which feed primarily on copepods (Hamner et al. 1982) and occasional, although relatively rare, *Aurelia* sp. 4 (Dawson & Jacobs 2001) jellyfish.

3.2 Data Collection

Environmental data, plankton samples and *M. papua* population surveys were collected every other month from 1999-2002, then monthly from 2003-2008 (Appendix II). Temperature, salinity, dissolved oxygen and pH were measured at meter intervals from the surface to below the chemocline using either a YSI 85 or a Hydrolab Quanta

meter. Environmental data were collected from the east and west basin and the mean of the mixolimnion (top 12 m; Martin et al. 2006) was used to estimate each environmental characteristic in the lake.

Plankton samples were collected using a 20 cm diameter, 80 μm mesh net, which was lowered past the chemocline, then sampled as a vertical haul from the chemocline to the surface. Plankton were collected in west, middle and east basins and preserved in 4% formalin for later identification and enumeration.

Plankton samples were concentrated into 15mL falcon tubes for storage. Before counting, the settled volume of plankton was recorded to determine the approximate seawater dilution amount to reach the optimum number of individuals to count (Harris et al. 2000). Samples were homogenized by gentle inversion at least twenty times before taking a 1 mL subsample with a Hensen-Stempel pipette and spreading it on a Sedgewick-Rafter cell. Samples were allowed to settle for approximately 10 min prior to enumeration. All zooplankton individuals and rare phytoplankton species were identified and enumerated by eye on the slide, to the nearest species or functional group possible, using a Leica S6D dissection scope. Larger phytoplankton species were numerous enough that a set of twenty photographs in a grid (representing 20% of the slide) were taken across the slide and identified and enumerated by eye from the photographs using the CellCounter tool in ImageJ (Schneider et al. 2012, De Vos 2001). The total number of phytoplankton species per subsample was then estimated by multiplying by the photographs' coverage area. If phytoplankton density was high enough at the initial dilution to make enumeration impossible, zooplankton were enumerated on the slide at the initial dilution, and a second dilution of the entire sample was done before photographing phytoplankton. Four subsamples were counted per basin per month. Subsamples and basins were averaged to produce an overall estimate and confidence interval for the lake per sampling date.

Mastigias papua population censuses were conducted by taking vertical hauls from the chemocline to the surface with half-meter diameter 1 mm mesh net in 15 locations arranged in a grid across the lake (a 'run'). Between one to three runs were performed for each sampling day. Visual surveys were conducted from March 1999 to September 2000 since *Mastigias* were either absent or too low to quantify. All medusae caught were measured to the nearest half-centimeter and tallied per size class per run. Totals per size class were averaged across runs and converted to density by dividing the total amount of water sampled per run, based on the location of the chemocline on the sampling day, as determined by the environmental data.

Pre-1999 values were calculated from published values of *Mastigias papua* population size from the late 1970s and from 1996 (Hamner & Hauri 1981, Dawson et al. 2001), and of environmental profiles from 1979, 1994 and 1997 (Hamner et al. 1982, Hamner & Hamner 1998). The pre-1999 zooplankton density plotted is the only published record for OTM, but it includes only the two copepod species in the lake. Therefore, this measure is representative of the largest proportion of zooplankton, but not total zooplankton density (from 1979, Hamner et al. 1982). All data included for the 'pre' estimates were collected prior to the ENSO event in Palau.

3.3 Data Analyses

Overall, I analyzed biotic and environmental dynamics using a monthly time step from 1999 to 2008. Months for which data were not available were treated as missing data in the analyses I subsequently performed. The environmental dataset included four variables (mean mixolimnion temperature, salinity, dissolved oxygen and pH) and the biotic dataset included three variables, *Mastigias,* zooplankton and phytoplankton densities, which were divided into subgroups (outlined below) for analyses. Although data collection enumerated all sizes and species present in the lakes, I excluded any variable (size class in *Mastigias* or species in plankton) which occurred in less than 10% of the months sampled due to low power.

Biotic data within the three main categories (level 1) were divided into subgroups (level 2), based on known feeding characteristics or interactions from other systems; actual species or size classes comprised level 3. Total *Mastigias* population density (level M1) contained the subgroup *Mastigias* ≥2 cm (level M2), which excludes (larval) ephyra recently strobilated from benthic polyps (Martin et al. 2006). Total zooplankton (Z1) was further subdivided into two categories based on the feeding methods of rhizostomes, which entrain prey in the fluid vortices created from bell pulsation (Costello & Colin 1995, D'Ambra et al. 2001). Without knowing the specifics of feeding and flow regimes for *Mastigias,* I grouped zooplankton into two general categories based on their potential avoidance reactions to a flow regime (or lack thereof; Waggett & Buskey 2006): Copepod density (Z2a) includes actively-escaping, post-naupliar copepods from two species of copepod in the lake, a cyclopoid copepod, *Oithona oculata,* and a calanoid copepod *Bestiolina inermis* (formally *Acrocalanus inermis*) (Hamner et al. 1982). The second subgroup is the remaining Passive prey (Z2b), which included nauplii of the two copepod species, bivalve larvae, veligers, fish eggs, trochophores and nereid larvae. Some of these species do have escape responses, but their escape velocities fall below the velocity threshold in other rhizostomes (Costello & Colin 1995, D'Ambra et al. 2001, Waggett & Buskey 2006). Total phytoplankton density (P1) was subdivided into Total dinoflagellates (P2a), which was comprised of three species of *Ceratium: C. inflatum, C. trichoceros,* and *C. teres*, and Total diatoms (P2b). All biotic data were ln(X+1) transformed before modeling.

Average monthly mixolimnion environmental and biotic data were analyzed for associations between groups using Generalized Additive Mixed Models (GAMM) (Hastie & Tibshirani 1990, Wood 2004, 2006). I tested for the order of autocorrelation in my data using acf() in R (stats, R Development Core Team), which indicated that two autocorrelation terms were appropriate for the data and no seasonality was present. I tested this indication of autocorrelation order in my data by building GAMM models with p=1, 2 and 3 and statistically comparing the models returned, using the anova() function in R on the lme item returned in gamm. The models constructed with autocorrelation order p=2 were either better than, or statistically indistinguishable from, those with an order of 1 and 3, so all final GAMM models were run with an autocorrelation order of 2. I also performed preliminary tests on the sensitivity of my model to the smoothing component dimension, k. As a general guideline, k should be large enough to provide reasonable assurance to represent the underlying patterns well, but small enough to

maintain computational efficiency, although the exact value of k is often not critical (Wood, 2004). I tested a range of values of k and then compared the resulting models as I compared models for autocorrelation. For most of my data, the exception being species or size classes that occurred rarely, there was little gain in increasing k after k=15. I set k=20 to be conservative, and adjusted it downward only in the case of testing rare groups that did not have the degrees of freedom to support that value.

To limit the loss of explanatory power from multiple tests, I adopted a two-step analysis approach often used in Path Analysis to target the interactions that were biologically relevant for testing. I first tested an abbreviated dataset, which included level 1 and 2 biotic groups and the environmental data. I targeted interactions between all environmental variables and the biotic groups, all net phytoplankton against zooplankton pairs, and both net phytoplankton and zooplankton against *Mastigias.* GAMM models were run for each pair using gamm() in R (mgcv, Wood 2004), with smooth terms constructed with k=20, or decreasing incrementally by half if there was insufficient data to support k, and using corARMA (nlme, Pinheiro et al. 2009) with p=2 to account for autocorrelation in the data. Models were run using equivalent and one-month lagged dependent variables and evaluated using AIC to determine the model chosen for each pair. The significance of the smoothing variable and the variation explained (R^2_{adj}) were recorded. Significance was evaluated after Sequential Bonferroni correction (Rice 1989).

In the second step of the analysis, any pair which had significant predictions was expanded to include its level 3 component parts (for example, a test of temperature v. *Mastigias* would be expanded into temperature against each *Mastigias* size class). This step was executed to see if any particular component of the group was driving the relationship, or the group as a whole. Significance of the extended dataset was evaluated after Sequential Bonferroni correction (Rice 1989).

GAMM models were plotted on the graphs by using predict() (R Development Core Team 2010) on the GAMM model across the span of the x axis. The model and standard error were then plotted on a scatterplot of the original data. The first derivative of the model (Deriv(), Simpson, 2011) was used to highlight parts of the curve with a slope significantly different from zero indicating a significant increase or decrease (colored blue or red). I plotted the density of *Mastigias* as a colored heatmap on any scatterplot which did not explicitly include *Mastigias* density as a variable, using the functions colorRampPalette() {grDevices} and findInterval() {base} in R (R Development Core Team 2010).

4 Results

The 1997/8 El Nino-La Niña event coincided with unusually hot, saline, oxygen rich water in OTM (Figure 1). Most environmental variables slowly returned to pre-perturbation levels over approximately two years (Figure 1). *Mastigias papua* population density remained low until the population recovery in 2000, after which it was high and variable until mid-2007, when it returned to pre-perturbation levels (Figure 1). A large component of the high population was medusae under 2 cm bell diameter. Microplankton densities in the lake were variable throughout the time period, without apparently

experiencing either a complementary population decline from the ENSO event, nor their highest values. Both zooplankton and net phytoplankton had higher mean values from 1999-2002 than the mean value for the rest of the time period, although only zooplankton was significantly different (means 0.065, 0.033; $p < 0.001$). Across that boundary, mean passive zooplankton, driven by copepod nauplii and bivalve larvae, significantly decreased, while copepods, driven by *Oithona oculata,* significantly increased (Table 1).

The association network of level 1 and 2 groupings indicates that environmental variables are significant explanatory variables for *Mastigias* population density; all but pH were significant for both of the *Mastigias* categories (Figure 2). Temperature was the strongest predictor, explaining 0.67 of the variance in *Mastigias* population density; dissolved oxygen was the next strongest, but the correlation between temperature and dissolved oxygen may have conflated the number. The dinoflagellate group was the only other biotic group associated with environmental variables, namely salinity, over the time series. The other significant associations between functional groups were between *Mastigias* and dinoflagellates, adult copepods and passive prey, and between zooplankton groups and diatoms. Association between biotic groups generally had less explanatory power than environmental groups.

GAMM models for the significant comparisons between level 1 and 2 functional groups shown as network connections in Figure 2 illustrate the complex association between the network nodes (Figure 3). Temperature and dissolved oxygen have little effect on *Mastigias* population density, up until a critical threshold is crossed (Figure 3 C, D), whereas increased salinity correlates with a steady decrease in *Mastigias* population density (Figure 3 B). Correlations between *Mastigias* and microplankton (Figure 3, middle row), illustrates negative correlations between all of them once *Mastigias* is present in the lake. Only the interaction between dissolved oxygen and temperature has an association with *Mastigias* population density (Figure 3 H); the other associations between microplankton show that *Mastigias* population density is haphazardly distributed throughout the plots.

Expansion of the association network to level 3 groups for any significant association in the level 1 and 2 network reveals that, generally, significant associations were driven by only one species or a few size classes (Figure 4). *Mastigias* size classes between 2 and 12 cm bell diameters account for the majority of the variation with environmental factors (Table 2). Larger *Mastigias*, between 7-17 cm bell diameters, are driving the patterns between zooplankton components, however. *Mastigias* association with passive zooplankton components are fairly evenly spread between the representative parts. Conversely, the interaction with adult pelagic copepods is almost entirely driven by interactions with *Oithona oculata,* not *Bestiolina inermis* (Table 2, Figure 4; for differences in *O. oculata* and *B. inermis* densities through time, see Appendix I). *Mastigias* size classes with significant interactions between a variable tend to follow the same overall pattern of association as the total *Mastigias* model of that variable, albeit with slight deviations (Figure 5). While many interactions follow simple positive or negative interactions, many others have shifts in their interaction relationship as densities change (Table 2).

5 Discussion

Uncertainties surrounding the mechanisms that influence scyphozoan-dominant systems are partly due to the complex and open systems in which scyphozoans occur. Using a marine lake as a natural closed-system microcosm, I was able to study the processes controlling fluctuations in a single population through time and the processes governing interactions between species in the medusae-dominated pelagic plankton community. *Mastigias* population size was higher and more variable, fluctuating fifty-fold interannually, following the 1997/8 El Niño-La Niña event than the population size was prior. Zooplankton densities were significantly higher during the period when *Mastigias* was absent and recovering than when *Mastigias* population densities were high. *Mastigias* is strongly affected by environmental conditions, and in turn, has a negative association with the prey items in the lake. Temperature and salinity were associated most strongly with *Mastigias* population size, with generally consistent associations across *Mastigias* size classes. There are moderately strong associations between zooplankton densities and the larger size classes of *Mastigias*.

5.1 Processes controlling *Mastigias* population size

Environmental variables, more than any biotic group, were most strongly associated with changes in *Mastigias* population size. The variable that explained the greatest variance was temperature, followed closely by the temperature-related dissolved oxygen and then salinity. Furthermore, the biotic groups that did associate with *Mastigias* population size displayed negative associations indicative of top-down control by the medusae, not bottom-up control of the medusa population. Thus, environmental forcing is the most likely process controlling *Mastigias* population size.

The association of low *Mastigias* population size with increased temperature had been previously established (Dawson et al. 2001, Martin et al. 2006) on a shorter time-series of these data. GAMM models reveal that the association between *Mastigias* population size and temperature is not linear, but instead has an abrupt threshold. *Mastigias* population densities hold fairly steady as temperature increases until ~31.25°C, after which the population falls precipitously.

Notably, temperatures higher than 31.5°C, approximately the temperature threshold in the association model, were associated with incremental decreases in *Symbiodinium* density in scyphistomae (benthic polyp stage) and a decreased frequency of strobilation (producing ephyrae, juvenile medusae)(Dawson et al. 2001). The association models do not necessarily mean that the environment is directly acting on the medusae stage of *Mastigias*; given the threshold temperature effects on the scyphistomae (Dawson et al. 2001), the association seen in the medusae could be a down-stream effect rather than a direct effect. Unfortunately, scyphistomae are difficult to find to do complimentary monitoring of their abundance and strobilation during perturbation events.

Dissolved oxygen (dO) concentration was highest in the lake when the lake was warmer and the medusae were absent, a seemingly counter-intuitive result. It is possible that the strong association of low dO concentration and high medusae number is

reflective of the association of dO with temperature, and temperature with *Mastigias*; multi-parameter GAMM models would be able to test this hypothesis. However, dissolved oxygen is an environmental variable that is intricately tied to the presence of respiring organisms. Although *Mastigias* is zooxanthellate, the photosynthetic output of oxygen from symbiotic *Symbiodinium* does not fully meet the respiration requirements of many medusae, especially smaller individuals (McCloskey et al. 1994). Thus, lower dO concentrations when there are higher concentrations of *Mastigias* could also be a result of several million medusae respiring in the lake rather than a cause of higher population size. In either instance, the seemingly counter-intuitive association is actually a reasonable and logical result.

5.2 Synecological consequences of *Mastigias* population fluctuations

Mean densities of plankton species were significantly higher when *Mastigias* was absent from the lake than upon the medusae recovery. Additionally, the models of *Mastigias papua's* associations with other microplankton in OTM displayed negative correlations indicative of top-down control. Taken together, these data suggest the associations between *Mastigias* and mesozooplankton are a predator-prey interaction. Mid- to large-sized, adult *Mastigias* had moderately strong and essentially equal associations with all of the 'passive prey' species and one copepod, *Oithona oculata.* These equivalent, broadly applied associations suggest that, if the associations are of predation, that *Mastigias* is a generalist feeder. The one exception is that there was limited association with *Bestiolina inermis*, the calanoid copepod, even though *B. inermis* and *O. oculata* occur in roughly equal (if alternating) abundances in OTM (Appendix I). *B. inermis* could have a higher escape velocity than *O. oculata* allowing it to not be caught by *Mastigias,* despite its prevalence in the lake.

Mastigias is zooxanthellate, but we know that the productivity rate of the *Symbiodinium* symbiont is variable and that some medusae, especially individuals smaller than 40 mm, must supplement their carbon intake, presumably with zooplankton prey (McCloskey et al. 1994). While there is no direct information on *Mastigias* diet, there are limited data from other rhizostomes. Comparison of a zooxanthellate and an azooxanthellate rhizostome (*Phyllorhiza punctata,* the sister taxon to *Mastigias,* and *Catostylus mosaicus*) indicated that both caught gastropod larvae, bivalve larvae and copepod nauplii on their oral arms (feeding appendages), although the zooxanthellate medusae caught proportionally more nauplii (Peach & Pitt 2005). Stomach contents of a rhizostome (*Stomolophus meleagris*) in the Gulf of California showed that fish eggs, bivalve larvae and copepods made a significant fraction of their diet (Padilla-Serrato et al. 2013). While we must not draw too many conclusions based on other species, these data do show that rhizostome medusae, including zooxanthellate medusae, are able to catch the kinds of prey which occur in OTM. Thus it is not unreasonable to tentatively attribute the changes in abundance of microplankton to predation by *Mastigias.*

Yet, the planktonic community's associations are more complex than *Mastigias* predation alone can explain. Some associations display density dependent switches in directionality (Table 2). Additionally, although population densities of microzooplankton were higher when *Mastigias* were absent, the densities were not higher than densities

reached at other points in time. It is possible that the filter feeders lining the mangrove roots on the side of the lake, which would comprise only a small proportion of the total biomass relative to the *Mastigias* when they are present, have a greater impact when *Mastigias* densities are low. There were minimal associations between microzooplankton species. The sampling does not capture what are probably highly abundant protists, smaller diatoms and other smaller primary producers that have been documented in the lake (Hamner et al. 1982) that we might expect to be associated with primary consumers in the microzooplankton. Even for models which had significant effects, there is still a lot of unexplained variation, suggesting other potential factors not incorporated in these models, or combinations of factors, are needed to fully explain variations in population sizes within a community. Hence, there is a need not only to continue to focus on the impacts of environmental variability on a single actor, but to focus on the linkages and feedbacks in the network, which can lead to highly complex, nonlinear and sometimes abrupt responses (Walther 2010).

5.3 Broader Implications

The factors driving scyphozoan population dynamics are poorly understood (West et al. 2009, Condon et al. 2013), as are their potential responses to environmental variability (Brotz et al. 2012, Condon et al. 2013). The uncertainty lies partly with the dynamic, open pelagic system in which scyphozoans occur, and partly due to a limited understanding of the geographic scales of scyphozoan populations. The factors that influence population fluctuations in medusae, and the top-down or bottom-up processes that shape their community have been unclear. Studying a distinct and complete population through time allows us to explore these factors more robustly. Although I found *Mastigias* exerts top-down predation pressure on microzooplankton, this result does not mean that bottom-up processes do not occur in other systems. It is possible that zooxanthellate medusae are decoupled from the bottom-up processes that occur in other systems due to a decreased reliance on zooplankton prey. I also found environmental forcing drives the medusae population through a threshold temperature after which the population drastically declines. There are potentially large implications depending on if species abundances steadily increase and decrease with changing environmental conditions, or if there is little variability in abundances as conditions change until a trigger point at which there, are sudden and large species responses. By studying one single scyphozoan population in a microcosm for long periods of time, we can start to tease apart the causal links driving medusae population dynamics and the consequences of those dynamics in a community.

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7 Tables & Figures

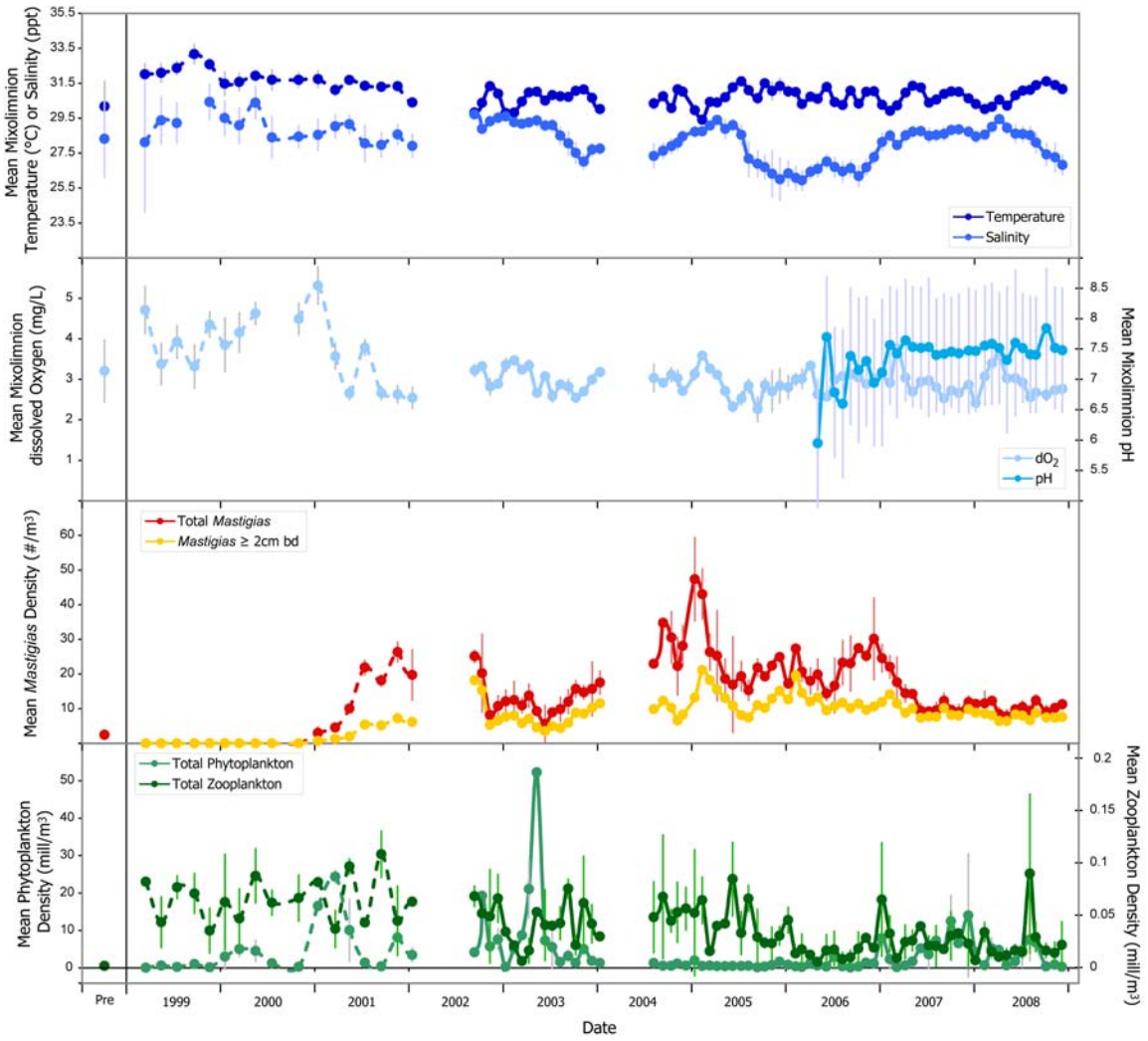


Figure 1. Ten-year time series of environmental (blues), *Mastigias papua* (reds) and microplankton (greens) population densities from March 1999 - December 2008. Solid line indicates monthly sampling; dashed line indicates bimonthly sampling. Error bars are 95% confidence intervals between replicate samples. Pre-1999 values were calculated from published values from the late 1970s and 1990s, prior to the ENSO perturbation event. Temperature (dark blue), salinity (blue), dissolved oxygen (light blue) and pH (teal) are the mean value across the mixolimnion (top 12 meters always above the chemocline). Medusae data is of the total medusa population density (red) and the total mean of individuals ≥ 2 cm bell diameter (bd; yellow). Values for temperature and *M. papua* before 1998 are given by the red arrows on axes (Dawson et al. 2001). Total phytoplankton (light green) and zooplankton (dark green) are the mean density above the chemocline, aggregated across species.

Table 1. Mean density of microplankton (millions m^{-3}) when *Mastigias* medusae are at low density from March 1999 until January 2002 compared to post-September 2002, when *Mastigias* population density is higher. Asterisks mark significant differences between absolute densities in the two time periods, after a tablewide Sequential Bonferroni correction.

Category	Functional Group & Components	Mean Density (mill/m^3) Mar 1999 – Jan 2002	Mean Density (mill/m^3) Sep 2002 – Dec 2008	*p*
Zooplankton	Copepods	0.027	0.016	*
	Oithona oculata	0.013	0.008	*
	Bestiolina inermis	0.014	0.008	
	Passive Zooplankton	0.038	0.017	*
	Copepod nauplii	0.028	0.009	*
	Bivalve larvae	0.008	0.003	*
	Veliger	0.0014	0.0009	*
	Egg	0.001	0.004	*
	Trochophore	0.0002	0.000	
Phytoplankton	Dinoflagellates	4.726	3.976	
	Ceratum inflatum	3.418	2.999	
	C. trichoceros	1.167	0.843	
	C. teres	0.141	0.133	
	Diatoms	0.003	0.001	

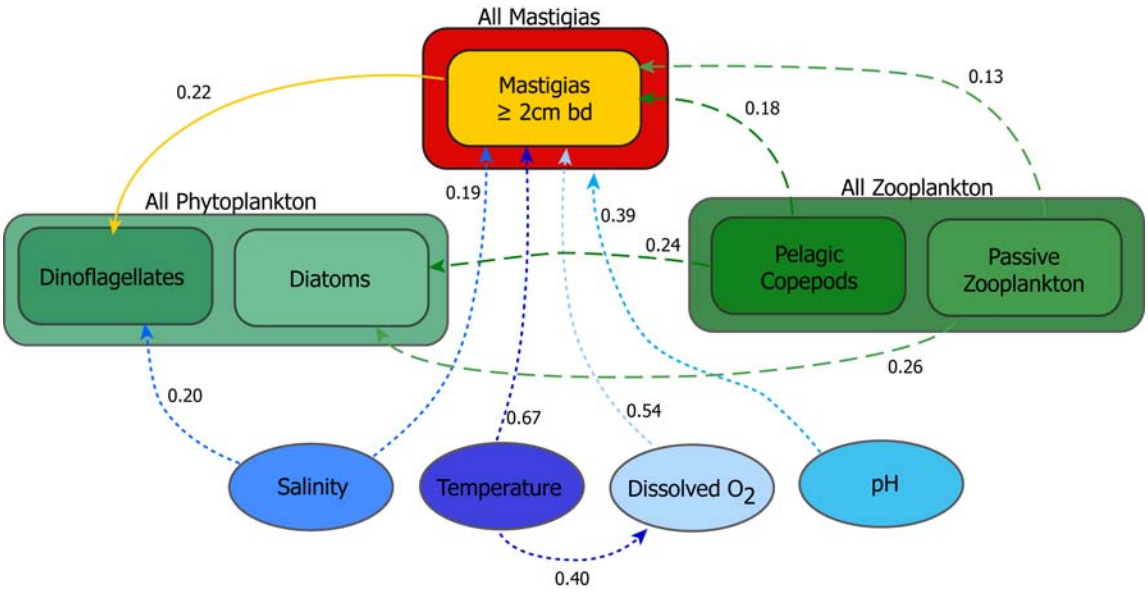


Figure 2. Association network, as constructed by GAMM modeling of level 1 and 2 functional groups and environmental variables across the ten-year time series in OTM. Circles are environmental variables; rectangles are biotic groups. Nested rectangles indicate level 2 subgroups within a level 1 functional group. Connecting lines illustrate significant explanatory power of the smooth term constructed from the independent variable of the dependent variable, after tablewide Sequential Bonferroni corrections. Arrows start at the independent variable and end at the dependent variable of the comparison; the direction of the relationship is given in Table 1. Numbers indicate the R^2_{adj} value. Lines are significant for all categories they pass through, although the numbers displayed are only for the comparison between the two ends of the line. Solid lines: from *Mastigias,* dotted lines: from environmental variables; dashed lines: from microzooplankton. Colors as in Figure 1.

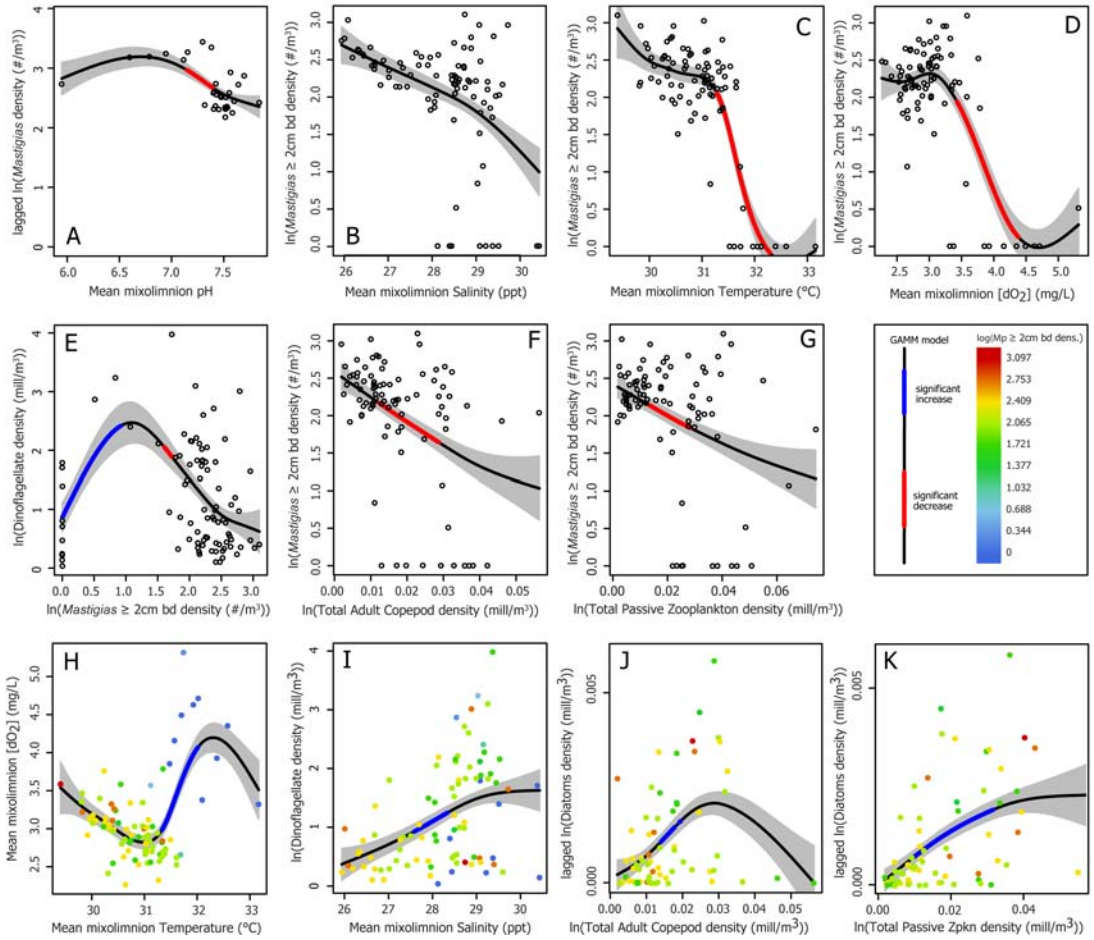


Figure 3. GAMM models of the significant interactions of the initial functional group comparisons, after Sequential Bonferroni corrections, showing the predicted model (solid black line) and standard error (grey shading). Circles indicate raw data points. Significant changes in slope, as calculated from the derivative of the GAMM model, are highlighted for significant increases (blue) or decreases (red) over the GAMM model line. For comparisons that did not include *Mastigias papua* population density explicitly, *M. papua* density is given as a heatmap over the scatterplot points. Comparisons between microzooplankton do not show indication of an underlying pattern due to medusa population density. The GAMM model between dissolved O_2 and temperature (bottom left) illustrates that hot, saline water was associated with lower *M. papua* population densities, consistent with other analyses by Dawson et al. (2001) and Martin et al. (2006).

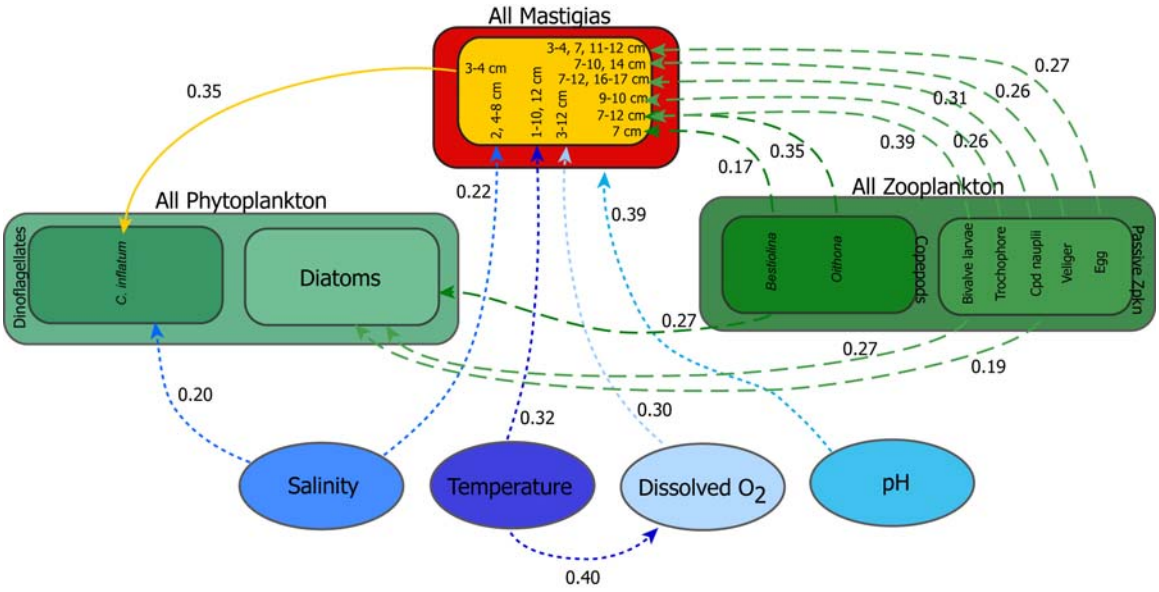


Figure 4. Expanded association network showing level 3 species/size class significant associations expanded from the significant associations in Figure 2. Values indicate R^2_{adj} for a given comparison or the mean R^2_{adj} if the arrows connect to a range of size classes. Colors, lines and arrows as in Figure 2.

Table 2. Comparisons between level 1 and 2 functional groups and any of their level 3 component parts which were significant. *Mastigias* size classes are given as a range of one centimeter size classes, if there was more than one that was significant. R^2_{adj} values are given as a min-max range if the comparison encompassed more than one comparison. Relationship describes the shape (∩,∪) or the pattern (- = negative, + = positive, s = stable) of the GAMM model. The order of terms indicates the fluctuation of the line.

Independent Variable		**Dependent Variable**			
Main	**Extended**	**Main**	**Extended**	**R^2_{adj}**	**Relationship**
Temperature		Dissolved Oxygen		0.395	- + -
Temperature		*Mastigias*		0.632-0.674	s -
			Mastigias 1-10, 12 cm	0.202-0.416	s -
Salinity		*Mastigias*		0.191-0.225	-
			Mastigias 2, 4-8 cm	0.172-0.283	-
Salinity		Dinoflagellates		0.196	+
Dissolved Oxygen		*Mastigias*		0.529-0.540	s -
			Mastigias 3-12 cm	0.242-0.437	s -
pH		*Mastigias*		0.394	∩
Mastigias		Dinoflagellates		0.222-0.279	+ -
			C.inflatum	0.281-0.352	+ -
	Mastigias 3-4 cm		*C.inflatum*	0.34-0.36	+ - s -
Adult Pelagic Copepods		*Mastigias*		0.183	-
			Mastigias 7-12 cm	0.192-0.366	U
	Oithona		*Mastigias* 7-12 cm	0.208-0.467	U
	Bestiolina		*Mastigias* 7cm	0.167	-
Adult Pelagic Copepods		Diatoms		0.237	∩
	Bestiolina	Diatoms		0.265	∩
Passive Zooplankton		*Mastigias*		0.132	-
			Mastigias 7-12, 15-17cm	0.175-0.444	-
	Copepod nauplii		*Mastigias* 7-12, 16-17cm	0.220-0.449	-
	Bivalve larvae		*Mastigias* 7-12cm	0.244-0.514	-
	Veliger		*Mastigias* 7-10, 14cm	0.182-0.303	U
	Egg		*Mastigias* 3-4, 7, 11-12cm	0.231-0.365	+ s -
	Trochophore		*Mastigias* 9-10cm	0.244-0.283	-
Passive Zooplankton		Diatoms		0.259	+
	Copepod nauplii	Diatoms		0.19	+
	Bivalve larvae	Diatoms		0.27	+ s

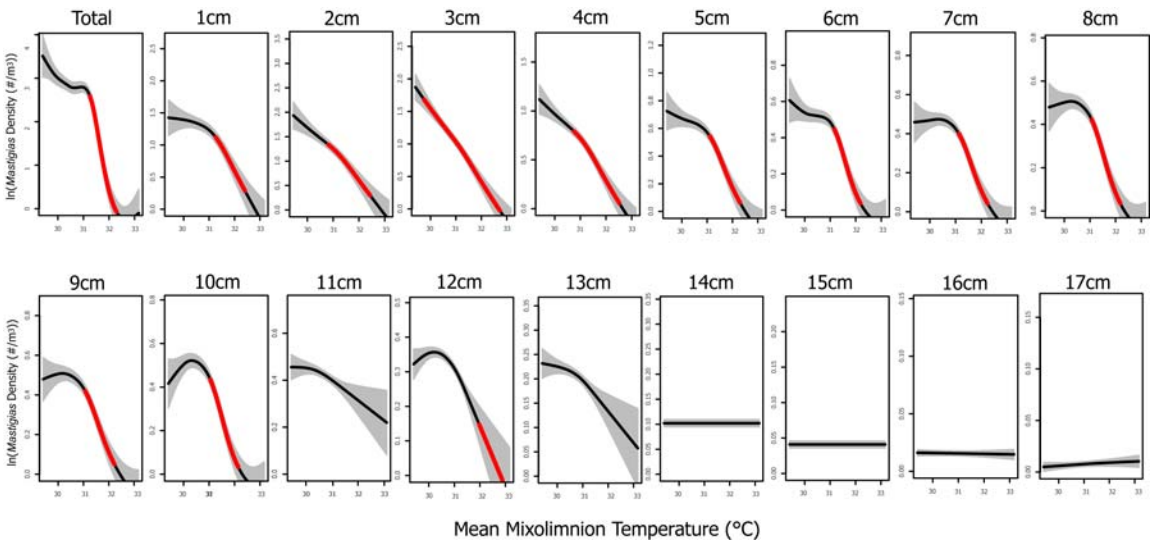
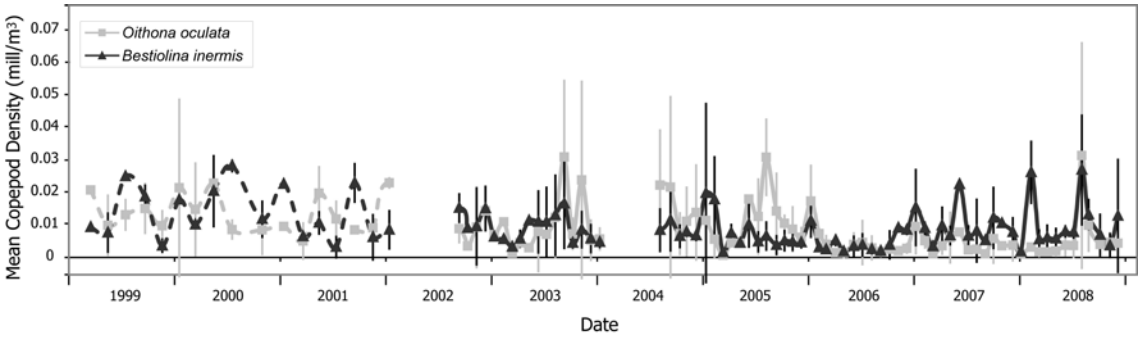
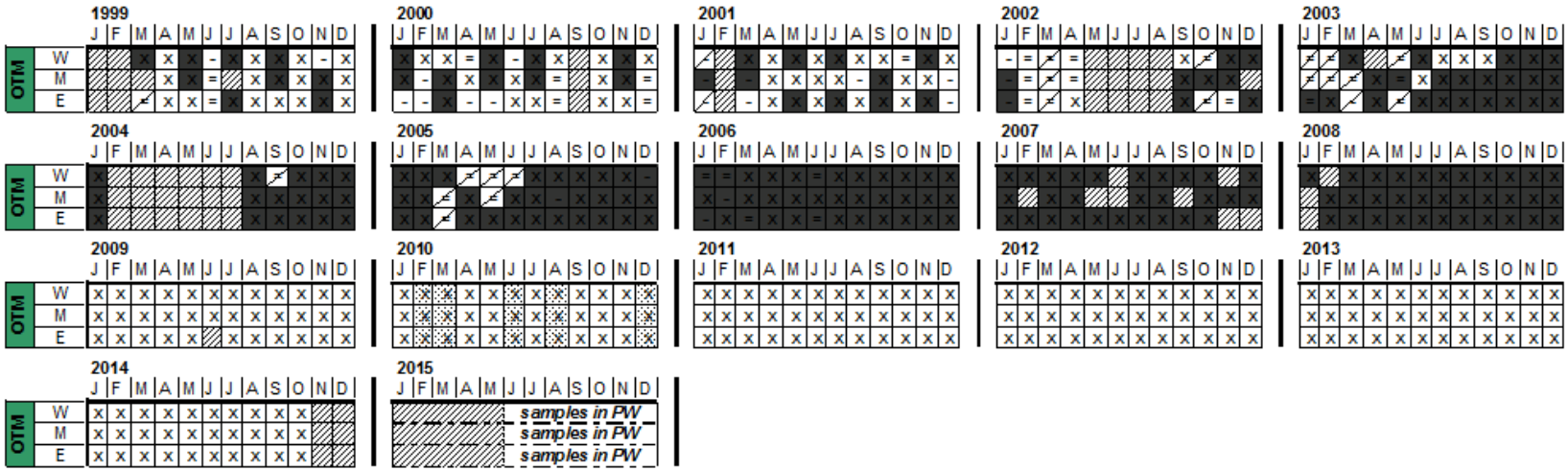


Figure 5. Example of *Mastigias* size class components compared to the overall model, mapped against temperature. Size classes 1cm - 10cm and 12cm were significantly explained by temperature. Although there are some small deviations between size classes significantly associated with temperature and the overall model, the pattern is consistent. Solid line, GAMM model; red overlay, significant decrease in model line; grey shading, standard error.



Appendix I. Mean densities (95% confidence interval error bars) of *Oithona oculata* (grey squares) and *Bestiolina inermis* (black triangles) across the ten year time series. Dashed lines indicate bimonthly sampling from 1999-2001.



LEGEND

Samples:

	Not Available
	Samples partially counted, but not included in dataset
black	Samples in dataset
x	Available sample
-	Partially dried out sample
=	Completely dried out sample
	Potentially biased from drying; not used

Appendix II. Microplankton specimens counted (dark shading), per basin and month in OTM. Sampling strategy was to count two basins per month, bimonthly from 1999-mid 2002, barring samples that were not collected or biased due to drying (dashes indicate dryness level). Samples between September 2002 and September 2003 were targeted to have at least two basins per month, barring unusable samples (diagonal lines). Post September 2002 through December 2008 was targeted to have as many basins as available counted.

CHAPTER 5:

CONCLUSION – APPLICATION OF ECO-EVOLUTIONARY DYNAMICS IN THE PELAGIC MARINE REALM

1 Summary of dissertation

Marine systems have historically lacked the microcosm framework of terrestrial and freshwater systems; microcosms act as 'natural laboratories' to better measure the relative ecological and evolutionary process acting within the system. My dissertation has utilized a marine microcosm to explore the evolutionary and ecological dynamics acting in the pelagic marine realm, specifically, the scyphozoan *Mastigias papua* and its plankton community. I did so by first establishing an evolutionary framework for *Mastigias spp.* across their range in the Indo-West Pacific, and then by examining single populations for ecological or evolutionary responses to extreme perturbation events.

Mastigias spp. in the Indo-West Pacific occur in two ecotypes – an ocean form and a marine lake form. I reconstructed phylogenetic relationships to establish three distinct species within the *Mastigias* species complex, leading to the inference that marine lake medusae are recently derived from independent colonizations from the ocean. Morphological crypsis in *Mastigias* has evolved through three patterns: morphological stasis between ocean medusae between species, convergence between lake medusae between species, and parallelism between lake medusae within species. In Chapter 3, I examined morphological and behavioral variation that occurred after a large perturbation event and subsequent medusae population crash and the potential impact of these variations on the community. I determined the evolutionary source of variation was endemic to the lake, either through differential expression of rare genotypes under varying environmental conditions or phenotypic plasticity. Finally, I explored the possible causes and consequences across a decade-long time series of a similar perturbation event and large population fluctuations of medusae. I found environmental forcing to cause fluctuations in medusae population size, and that those fluctuations could impact the planktonic community through top-down effects.

2 Application to eco-evolutionary dynamics

In terms of the eco-evolutionary dynamic, each chapter studies different, but complementary, aspects along evolutionary and ecological spatio-temporal scales (Figure 1). Chapter 2 established the evolutionary framework across micro- and macro-evolutionary scales by reconstructing the phylogenetic history of *Mastigias* over millennia (Figure 1A). I used genetics to define discrete populations on the local and

region spatial scales to understand the evolutionary patterns that create morphological (ecological) differences. Chapters 3 and 4 utilize the knowledge of broad-scale evolution and definitions of populations to examine finer-scales of resolution. Chapter 3 follows a perturbation event that caused a population decline (ecological) and triggered a plastic or microevolutionary response (evolutionary) (Figure 1B). We studied how those evolutionary sources of variation could have then caused variation in morphology and behavior in the population (ecological) and suggested potential impacts that might have on the plankton community. Chapter 4 focuses on ecological aspects of a similar perturbation and studies the processes causing the population abundance change and the impacts in the community (Figure 1C).

In each case, we have succeeded in expanding our knowledge about aspects of the eco-evolutionary dynamic and laying the foundation for fully completing the eco-evolutionary feedback loop. For example, in Chapter 3, we established the link between the lack of genetic change (evolutionary) and the morphological and behavioral differences (ecological) between demes. To complete the feedback loop, we would need to know to what part of the perturbation – environmental, population decline, or other factor – caused the evolutionary change that resulted in variation in medusae morphology and behavior. To understand any potential ramifications that might have on the community, we would need to know *Mastigias'* diet, and how that could change with changing morphology. We would also need to know the responses the planktonic prey have to the same perturbation event. These causal links are missing, but the dissertation lays the groundwork to expand and build upon with future work.

3 Future Directions

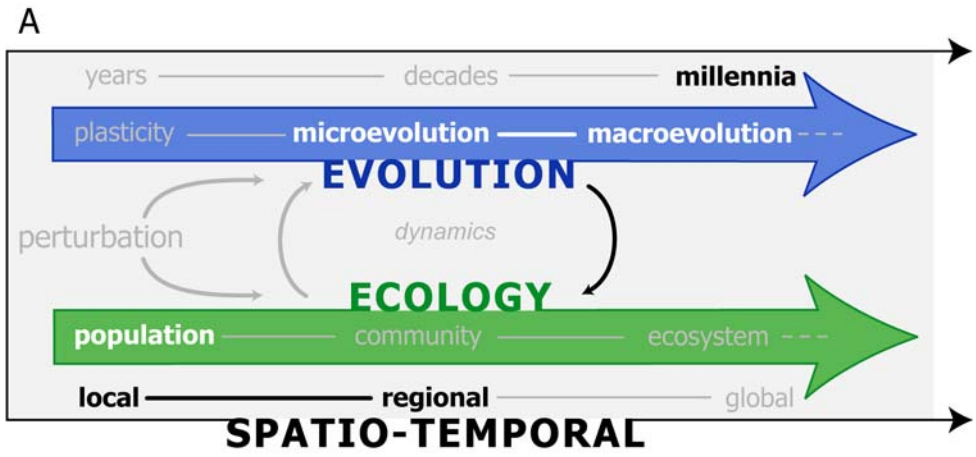
Untangling the eco-evolutionary dynamic requires long-term field experiments to address each of its components. Future directions should not only seek to add the causal links, but follow the same eco-evolutionary dynamic as it iterates through time. For example, it would be interesting to know if the variation seen in NLK leads to a rare genotype increasing in frequency in the lake as the perturbation ends and the medusa population increases, if potential plastic responses lead toward evolutionary canalization, or if the system returns to its pre-perturbed state and genotype frequencies.

Future work should also seek to apply the eco-evolutionary framework described in the first data chapter as a comparative framework to test hypotheses of evolution and ecology. For example, the population perturbations seen in OTM and NLK occur asynchronously, and even now, as the NLK population increases, the OTM population is declining. The comparative framework of the marine lakes, as described as an evolutionary framework in Chapter 2, can allow exploration of parallel, convergent, or divergent patterns that underlie seemingly similar changes. This framework spans multiple spatio-temporal, ecological and evolutionary scales, which is what makes it such a valuable tool.

4 Closing remarks

Ecological and evolutionary processes act concurrently, with constant feedback loops occurring. Even if a study targets only one direction of the dynamic, the entire history of the eco-evolutionary feedback loop that has already run is present and impacting the system. Thus, it is important to keep these interactions in mind when designing and executing studies. Comparative frameworks can elucidate patterns and processes, as well as aid in predictions of similar systems. As climatic variability increases, together with other aspects of climate change, it is increasingly important to understand the eco-evolutionary dynamic of systems and the potential impacts perturbations can have on them.

5 Figures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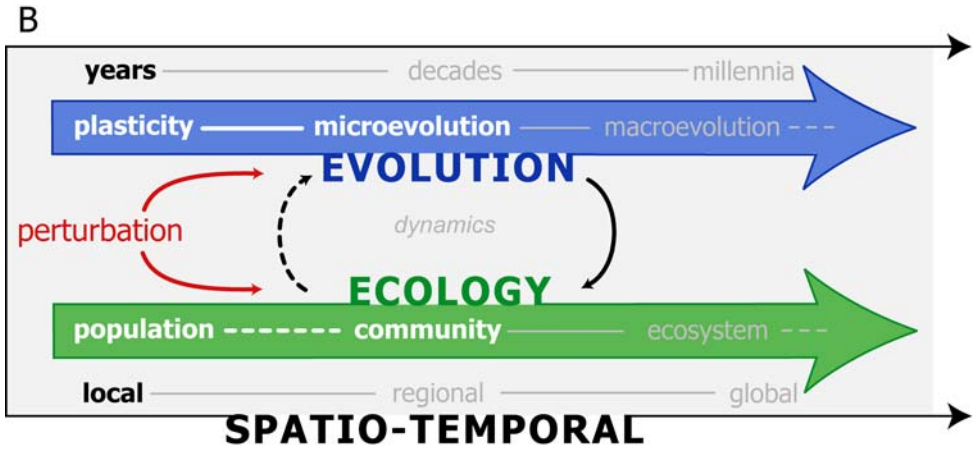
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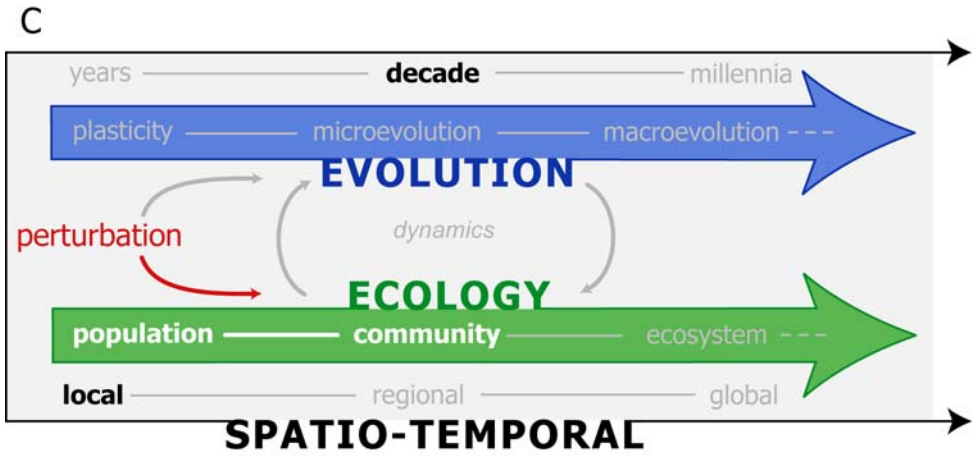
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Figure 1. Schematic of eco-evolutionary dynamics (as in Figure 1-2), with the scales and direction of the dynamic investigated in the dissertation. Bolded terms and arrows indicate the aspects of the dynamic studied in (**A**) Chapter 2, (**B**) Chapter 3, and (**C**) Chapter 4. Dashed lines indicate suggested impacts not directly measured.