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SANTA CRUZ

UNCHARTED WATERS: BIVALVES OF MIDWAY ATOLL AND INTEGRATING MATHEMATICS INTO BIOLOGY EDUCATION

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

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY
with an emphasis in EDUCATION

by

Kristin M. McCully

September 2013

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ABSTRACT

UNCHARTED WATERS: BIVALVES OF MIDWAY ATOLL AND INTEGRATING MATHEMATICS INTO BIOLOGY EDUCATION

By Kristin M. McCully

To protect and conserve the Earth's biodiversity and ecosystem services, it is important not only to understand and conserve species and ecosystems, but also to instill an understanding and appreciation for biodiversity and ecosystem services in the next generations of both scientists and citizens. Thus, this dissertation combines research into the ecology and identity of large bivalves at Midway Atoll in the Northwestern Hawaiian Islands (NWHI) with research on pedagogical strategies for integrating mathematics into undergraduate biology education.

The NWHI is one of the few remaining large, mainly intact, predator-dominated coral reef ecosystems and one of the world's largest marine protected areas. Previous bivalve studies focused on the black-lipped pearl oyster, *Pinctada margaritifera*, which was heavily harvested in the late 1920s, has not recovered, and is now a candidate species for restoration.

First, I combined remote sensing, geographic information systems, SCUBA, and mathematical modeling to quantify the abundance, spatial distributions, and filtration capacity of large epifaunal bivalves at Midway Atoll. These bivalves are most abundant on the forereef outside the atoll, but densities are much lower than reported on other reefs, and Midway's bivalves are unlikely to affect plankton abundance and productivity inside the lagoon.

Second, I used molecular techniques and phylogenetic reconstructions to identify pearl oysters (*Pinctada*) from Midway Atoll as *P. maculata,* a species not previously reported in Hawaii. As a small morphologically cryptic species, *P. maculata* may be a native species that has not been collected previously, a native species that has been identified incorrectly as the morphologically similar *P. radiata*, or it may be a recent introduction or natural range extension from the western Pacific.

Finally, I review science education literature integrating mathematics into undergraduate biology curricula, and then present and evaluate a computer inquiry module using learner-centered teaching strategies to introduce structured population (matrix) models in an upper-division ecology course. Using pre- and post-test surveys and student interviews, I concluded that students accomplished the module's learning goals: to use matrix models to assess and protect populations and to better appreciate the importance and uses of mathematics in ecology and conservation.

INTRODUCTION

To protect and conserve the Earth's biodiversity and ecosystem services, it is important not only to understand and conserve species and ecosystems, but also to instill an understanding and appreciation for biodiversity and ecosystem services in the next generations of both scientists and citizens. Thus, this dissertation combines research into the ecology and species identification of large bivalves at Midway Atoll in the Northwestern Hawaiian Islands with research on pedagogical strategies for integrating mathematics into undergraduate biology education.

Frequent lack of recovery of bivalve mollusk species after depletion due to harvesting has increased awareness of the many ecosystem functions and services provided by filter-feeding bivalves and led to restoration/repopulation efforts in many parts of the world. Ecosystem functions and services provided by bivalves include water filtration that enhances water quality by removing nutrients, plankton, and heavy metal pollutants, and the production and harvesting of wild and aquaculture bivalves for fisheries and jewelry. Because they remove plankton and suspended solids that block the sunlight necessary for photosynthesis in zooxanthellate reef-building corals and benthic algae, bivalves may be particularly important in coral reef ecosystems, including those of the Hawaiian Archipelago.

As one of the most isolated archipelagos in the world, in the middle of the world's deepest and widest ocean, Hawaii supports some of the world's highest levels of marine endemism. The Northwestern Hawaiian Islands are one of the few large-scale, largely intact, predator-dominated coral reef ecosystems left in the world. The mostly uninhabited, emergent islands, atolls and submerged banks in the northwestern part of the archipelago are protected as the Papahānaumokuākea Marine National Monument (PMNM) and a

World Heritage Site. The PMNM is now one of the largest marine protected areas on Earth and the largest conservation area in the United States. Due to its remoteness and regulations limiting access, current impacts from local human activities are small, but there are residual effects of past uses (e.g., military facilities) and distant anthropogenic impacts, such as global climate change and marine debris.

Despite the potential value of their ecosystem functions and services, very little information is currently available on the bivalves and other mollusks of the Northwestern Hawaiian Islands. The few previous studies focused mainly on the black-lipped pearl oyster, *Pinctada margaritifera*, which was heavily harvested in the late 1920s at Pearl and Hermes Atoll, has not yet recovered, and is now a candidate species for restoration. Other bivalves that potentially compete for space and planktonic food with *Pinctada margaritifera* are rarely mentioned.

In CHAPTER ONE, I quantify the abundance, spatial distribution, and filtration capacity of large epifaunal bivalves at Midway Atoll, the second northernmost reef in the Hawaiian Archipelago. I used remote sensing and geographic information system (GIS) tools to classify the atoll's shallow benthic environment into four main habitats and seven geographic sectors and then randomly selected between three and seven sites for belt transect surveys in each combination of habitat and geographic sector. I used statistical tools and mathematical models to analyze data from 108 belt transects surveyed with SCUBA. Two of the three common species, *Spondylus violacescens* and *Streptopinna saccata*, are most abundant on the forereef outside the atoll's rim, possibly because this habitat contains many overhangs and crevices providing refuge from predators. Although very few studies have quantified bivalve population sizes in coral reef habitats, the densities of bivalves at Midway are much lower than those reported at other coral reef locations. Estimates based on size-frequency distributions and population abundances suggest that these bivalves are unlikely to be able to affect plankton abundance and productivity inside the Midway Atoll lagoon.

In CHAPTER TWO, I use molecular techniques and phylogenetic reconstruction to identify pearl oyster (*Pinctada*) recruits collected at Midway Atoll as a species not previously reported in Hawaii. Thousands of marine species worldwide cannot be labeled as native or nonnative due to a lack of systematic, biogeographic, historical, and fossil data; such species, whose origins are unknown, are described as “cryptogenic”. Cryptogenic species and cryptic invasions are likely to be particularly common in species that are poorly resolved by external morphology, such as pearl oysters in the genus *Pinctada* (Phylum Mollusca, Class Bivalvia, Order Pteroidea, Family Pteriidae). I sequenced a variable internal transcribed marker, ITS1, from 17 Midway Atoll *Pinctada* juveniles and compared them with GenBank sequences for 11 *Pinctada* species and one outgroup, using maximum likelihood and Bayesian inference approaches to reconstruct the phylogeny of the genus *Pinctada.* In all phylogenetic reconstructions, the *Pinctada* recruits from Midway clustered most closely with *P. maculata*, a species not previously reported in the Hawaiian archipelago. As a small and morphologically cryptic species, *P. maculata* may be a native species in Hawaii that has not yet been collected, a native species that has been collected but incorrectly identified as a morphologically similar species that is reported in Hawaii, or an unreported, recently introduced species. None of the sequenced juveniles were *Pinctada margaritifera* which is known only from adults on Midway Atoll. This study highlights the importance of molecular techniques for identifying species and studying connectivity in conservation work.

Many recent national evaluations of undergraduate biology education, such as the 2003 report *BIO2010: Transforming Undergraduate Education for Future Research Biologists* and the 2011 report *Vision and Change in Undergraduate Biology Education: A Call to Action*, call for better integration of quantitative and mathematical modeling skills into biology curricula. Students need these skills to obtain a deep understanding of biological phenomena and to contribute effectively to future scientific inquiry. Strategies implemented by various instructors and institutions include: restructuring math courses for biology majors to incorporate biology examples; completely integrating freshman-level math and biology courses; creating quantitative biology majors with unique capstone courses, seminars, and/or research experiences; including quantitative skills such as statistical analysis and modeling in undergraduate research experiences; integrating quantitative approaches throughout the life sciences curriculum; and creating and using quantitative biology modules for easy integration into existing courses.

In CHAPTER THREE, I review science education literature about integrating mathematics into undergraduate biology curricula, and then present and evaluate a computer inquiry module using learner-centered teaching strategies to introduce structured population (matrix) models in an upper-division ecology course. The module consists of: (1) an interactive foundational lecture that introduces the concepts and mechanics of structured population models in the context of a conservation case study and (2) a computer inquiry laboratory in which students work in small groups to develop and analyze a structured population model about a specific population and then use it to ask and answer their own conservation questions about a specific populations. Using a pre- and post-test design and student interviews in two iterations of an upper division ecology course at a research university, I concluded that students accomplished the learning goals of the module: to use matrix models to assess and protect populations and to better appreciate the importance and uses of mathematical models in ecology and conservation. These conclusions and the students' comments are consistent with previous research indicating that learner-centered strategies facilitate active learning and increase student interest and motivation, allowing students to gain a deeper understanding of biology as a process of inquiry and learning of biological concepts. These strategies are also likely to support the approximately one-third of college students who have trouble with quantitative work because of “mathematics anxiety”.

CHAPTER 1

Distribution and Abundance of Epifaunal Bivalves at Midway Atoll, Northwestern Hawaiian Islands

Introduction:

Lack of recovery of several bivalve mollusk species after depletion due to harvesting has increased recognition of the many ecosystem functions and services provided by filter-feeding bivalves and led to restoration/repopulation efforts in many parts of the world. Ecosystem functions and services include: water filtration that removes nutrients, plankton, and heavy metal pollutants and maintains water quality; habitat construction for diverse invertebrates and fishes that increases species diversity and trophic complexity; stabilization of benthic or intertidal habitats that aids shoreline protection; sequestration of carbon; benthic-pelagic coupling of nutrient cycles; and production and harvesting of wild and aquaculture bivalves for fisheries, jewelry, and fertilizer (Coen et al. 2007, Dame 2012b). For example, the eastern oyster (*Crassostrea virginica*) is both an ecosystem engineer, creating hard substrate reefs, and a keystone species, exerting top-down control of plankton biomass (NOAA 2007).

Many studies, reviewed in Dame (2012a), show that native and introduced bivalves may have significant impacts on plankton communities and reduce the quantities of suspended solids and phytoplankton. Dame (2012a) argues that bivalves can control phytoplankton biomass in systems with high bivalve biomasses and long water residence times, such as South San Francisco Bay (California), Narragansett Bay (Rhode Island), Bay of

Marennes-Oléron (France), and the Oosterschelde estuary (Netherlands). Several bivalve species are also widely used for monitoring and bioremediation of excess nutrients, microorganisms, and heavy metals due to their sessile habits, massive pumping and filtering capabilities, and ease of transplantation and maintenance (Kimbrough et al. 2008, Gifford et al. 2007).

Despite the importance of bivalves in many marine ecosystems, there has been much less research on bivalves than on many other taxonomic groups. For example, coral reef research focuses mainly on Actinopterygii (ray-finned fishes) and Anthozoa (anemones and corals) and neglects bivalves (Fisher et al. 2011). Because they remove plankton and suspended solids that block the sunlight necessary for photosynthesis in zooxanthellate reef-building corals and benthic algae, bivalves may be particularly important in coral reef ecosystems, such as the Hawaiian Archipelago.

In addition to providing ecosystem functions and services, bivalves are important in the traditional Hawaiian culture as sources of tools, instruments, ornaments, and food, as evidenced by museum collections and middens associated with most archaeological sites (Severns 2011). Due to Hawaii's extreme isolation in the center of the world's largest and deepest ocean, Hawaiian marine mollusks include relatively few species that are all good long-distance dispersers and a relatively high percentage (~21%) of endemic species (Bouchet 2011, Severns 2011:22, Kay and Palumbi 1987).

Until very recently, very little information was available about bivalves at Midway Atoll and the other Northwestern Hawaiian Islands (NWHI). Defelice et al. (1998) reported that no systematic marine invertebrate survey had been conducted at Midway prior to their 1997 survey and that only 85 invertebrate taxa (of all phyla) from Midway were then in

Bishop Museum collections. In surveys at 12 sites over four days in 1997, mostly focusing on non-native species on artificial substrates, Defelice et al. (1998) found 316 invertebrate species, including 50 mollusks and 7 bivalve species. The National Oceanic and Atmospheric Administration (NOAA), U.S. Fish and Wildlife Service (USFWS), the University of Hawaii, and other agencies have since cooperated to send Rapid Ecological Assessment teams to assess the coral reefs of the NWHI in 2002, 2003, 2004, and 2008, but these teams spent only a few days at each reef surveying a limited number of sites with short belt transects. For example, all surveys to 2008 recorded a total of 6 bivalve species at Midway Atoll, and the six 2008 surveys included only 11 individual bivalves of 4 species at Midway (NOAA CRED, unpublished data).

The present study estimates the abundances, size distributions, and filtration capacities of large epifaunal bivalves in the shallow marine habitats of Midway Atoll, using randomly-located belt transects to surveys stratified by habitat and geographic sector. While bivalves often have major impacts on ecosystems by consuming large amounts of phytoplankton and thereby limiting primary production directly and secondary production indirectly (Gili and Coma 1998), they are only one group of suspension-feeders in the complex and diverse coral reef community (along with corals, sponges, ascidians, some fishes, and others). Because various suspension-feeders feed on different forms and sizes of plankton, each is important to the community (Gili and Coma 1998, Riisgård and Larsen 1995). Other large bivalves may be particularly important in the Northwestern Hawaiian Islands because they are potential competitors of the black-lipped pearl oyster, *Pinctada margaritifera*, which was heavily harvested in the late 1920s at Pearl and Hermes Atoll, has not yet recovered, and is a candidate species for restoration efforts (Keenan et al. 2006, Keenan 2007, PMNM 2008).

Materials and Methods:

Study Region: Hawaiian Archipelago

As one of the most isolated archipelagos in the world, in the middle of the world's deepest and widest ocean, Hawaii possesses some of the highest levels of marine endemism in the world (e.g., 24% of nearshore fishes, Randall 2007). The Hawaiian archipelago stretches over 2,500 km and 10 degrees of latitude from the island of Hawaii (19°34′N 155°30′W) to Kure Atoll (28°25′N 178°20′W) (Figure 1). The islands and reefs of the Hawaiian archipelago range in age from active lava flows on the southeast side of the island of Hawaii to seven million-year-old Kauai, and then out to Midway and Kure Atolls (~28 million years old) (Friedlander et al. 2008a,b). The Hawaiian archipelago consists of two regions: the eight Main Hawaiian Islands (MHI) that are populated, high volcanic islands; and the Northwestern Hawaiian Islands (NWHI) including mostly uninhabited, emergent islands, atolls and submerged banks. Reefs in the MHI are under tremendous pressure from 1.3 million residents and nearly 7 million tourists each year and suffer from many land-based sources of pollution, overfishing, recreational overuse, and introduced species (Friedlander et al. 2008a).

At the other end of the archipelago, the NWHI reefs form the Papahānaumokuākea Marine National Monument (PMNM) and World Heritage Site, which is co-managed by NOAA, USFWS, and the State of Hawaii. As one of the largest marine protected areas on Earth and the largest conservation area in the United States, the PMNM encompasses

362,061 km^2, an area larger than all U.S. national parks combined (Figure 1, PMNM 2008). The NWHI are one of the few large-scale, largely intact, predator-dominated coral reef ecosystems left in the world. The NWHI reefs also have extremely high proportions of endemic species across many taxa (e.g., 30% of coral and shallow water reef fish species and much higher percentages based on abundance) with virtually no impacts from alien species (Friedlander et al. 2008b, Grigg et al. 2008, Maragos et al. 2004). Due to the monument's remoteness and regulations that limit access, current impacts from local human uses are small, but there are residual effects of past uses, such as the Pacific Cable Station and the U.S. Naval Air Facility Midway Island (1941-1996). Distant human impacts, such as global climate change and marine debris, are now the main human impacts on the monument's coral reefs.

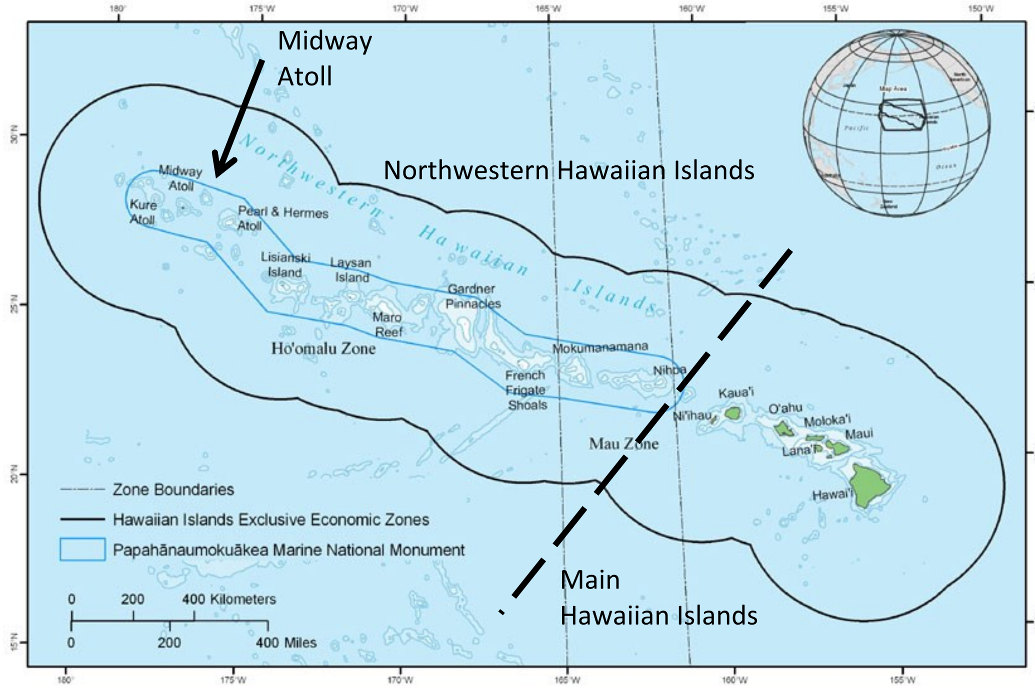

Figure 1: Hawaiian archipelago with Exclusive Economic Zone (black lines), Papahānaumokuākea Marine National Monument borders (blue line), and location of Midway Atoll (PMNM 2008).

Winds, storms, waves, currents, and water temperatures strongly affect the biota and structure of coral reef ecosystems in the Hawaiian Archipelago. In the NWHI, tradewinds from the northeast and east prevail for much of the year, while winter storms from the northwest and west create large waves that may break as deep as 20 m. These waves both produce concussion forces and re-suspend sand and other particulate matter that abrades and scours bottom communities (Grigg et al. 2008). Thus, the barrier reef is poorly developed or absent from parts of the northwest sides of Pearl and Hermes Atoll, Midway Atoll, and Kure Atoll, and there is very low coral cover (5-10%) on the north and west forereefs. Shipboard Acoustic Doppler current profile data shows that, despite great variability, mean flow of surface waters throughout the NWHI is predominately from east to west driven by the prevailing northeasterly tradewinds, with predominantly southwestward flows around Kure, Midway, and Pearl and Hermes Atolls (Firing et al. 2004, Firing and Brainard 2006). Most reef biota, either as larvae or attached to debris, probably reached Hawaii via the east-flowing Subtropical Counter Current from the Indo-West Pacific via Johnston Atoll (Fletcher et al. 2008, Kay and Palumbi 1987, Maragos et al. 2004). Although summer water temperatures are similar along the island chain, with a peak of about 28°C, the northern islands experience much lower winter temperatures, with peak lows of 17-18°C, than the southern islands, with peak lows of about 22°C, and thus much greater annual variation in temperature (10°C vs. 5°C) (Grigg et al. 2008, Potts et al, unpublished data).

The exact percentage of endemic marine mollusks in Hawaii is unknown due to the lack of good data on species presence and distributions, but about 21% of marine gastropods are endemic; this is the highest estimate of endemic mollusk species recognized for any Pacific island group (Severn 2011:22, Kay & Palumbi 1987). In the first comprehensive guide to Hawaiian marine mollusks, E. Alison Kay recognized 966 marine shelled mollusks in her landmark *Hawaiian Marine Shells* in 1979. More recently, Severns (2011) listed 1333 marine shelled mollusks.

Study Area: Midway Atoll

The study was carried out in the shallow marine environments of Midway Atoll at 28°12′N 177°21′W (Figure 2). Midway is a roughly circular, emergent atoll with three small islets (Sand, Eastern, and Spit) on the southern side of the lagoon and a shallow opening on the northwest side. It is 10.6 km long and 9.3 km wide with a total reef area of 348 km^2 (PMNM 2013). The lagoon within the atoll's reef rim has an area of about 70 km^2 (an imprecise estimate because the lagoon is not fully enclosed). Mean depth is 3 m (calculated from NOAA 2003), with maximum depth inside the lagoon of nearly 30 m (McCully, pers. obs.). Midway is about 2,100 km west-northwest of Honolulu in the MHI, just 260 km east of the International Dateline, and nearly halfway between San Francisco and Japan.

Midway Atoll is currently protected and managed as the Midway Atoll National Wildlife Refuge, a Special Management Area within the PMNM. Although it has experienced little fishing and has an intact trophic structure, over 50 years as a large Naval Air Facility impacted the coral reef ecosystem by extensive engineering, including dredging the lagoon and harbor for anchorages, cutting the channel through the south rim, removing patch reefs for seaplane runways, and greatly increasing the area of the main island (Figure 2). Midway is the second northernmost atoll in the world and one of the most northerly coral reefs in the world, so winter water temperatures are marginal for most coral reef biota.

Figure 2: Satellite IKONOS image of Midway Atoll.

Stratification of Survey Areas

To maximize efficiency and precision of population estimates, I conducted random stratified sampling by dividing the atoll's shallow marine habitats into 4 habitats and 7 geographic sectors (Figure 3, 4). Using NOAA's (2003) classification of 21 benthic habitats based on satellite imagery, I grouped the atoll's shallow-water habitats, at 4 m x 4 m pixel size, into 4 general, common habitat categories based on the substrate type and location relative to the atoll rim (Figure 3, Table 1): Forereef, Backreef, Patch Reef, and Sand. I excluded the two piers on the northeast side of Sand Island and the inner boat harbor because these sites are primarily artificial substrates and very different from other benthic habitats, although they do support large numbers of bivalves. To maximize survey time while scuba diving, survey sites were restricted to depths of ≤ 20 m on the forereef, using bathymetric data in NOAA (2003).

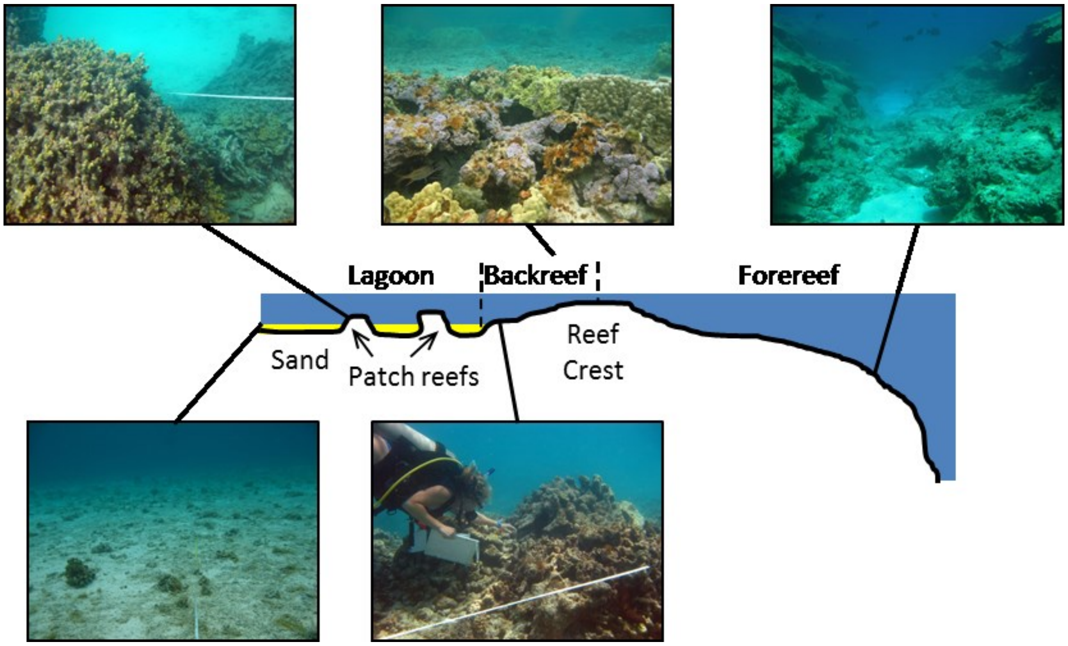

Figure 3: Diagram of coral reef habitat distribution in an atoll, with photos of typical sites from Midway.

Table 1: Summary of habitat categories and identifying characteristics.

Habitat (Area)	Depth	Description
Forereef ($11.4\ km^2$)	5-20 m	Located outside atoll's reef rim. Most exposed to storms and wave energy. Spur and groove formations often provide 3-8 m of vertical relief and often include overhanging ledges and channels of sand at bottom of grooves. Sharks common. Kenyon et al. (2010) report average of 1.6% coral cover, with predominantly encrusting and massive *Porites* and some *Pocillopora*. This is consistent with my observations.
Backreef ($25.4\ km^2$)	1-6 m	Mostly hard substrate located just inside atoll's reef rim. Coral cover can reach up to 40% in areas on north and east sides with diverse species, including *Porites lobata, Montipora turgescens/flabellata, M. capitata, Pocillopora meandrina,* and *P. damicornis*. Kenyon et al. (2010) reports average coral cover peaks with 15.2% in north backreef, but remains >5% and is dominated by *Montipora turgescens/flabellata* throughout the NW to E sectors. In the SE to W sectors, coral cover ranges 1-3% and is dominated by *Pocillopora* and massive and encrusting *Porites* (Kenyon et al. 2010). This is consistent with my observations.
Patch reefs ($3.1\ km^2$)	2-8 m	Reefs of mostly dead *Porites compressa* and *P. lobata* surrounded by sand inside the atoll's lagoon. Kenyon et al. (2010) reports 1.7% coral cover on these patch reefs. Occasional areas of live *Porites compressa*, but main coral is occasional heads of *Pocillopora meandrina*. Some patch reef sites are mostly rubble. Schroeder and Parrish (2006) give detailed descriptions of patch reefs in the southwest lagoon, known as Welles Harbor.
Sand ($49.3\ km^2$)	2-6 m	Very flat with varying amounts of rubble and algae on top of sand inside the atoll's lagoon. Occasionally covered by seagrass (*Halophila hawaiiensis* or *H. decipiens*). Almost no coral and very few fish. Occasionally reached 16 m deep.

To distribute survey sites evenly over the geographic area of the atoll and optimize precision, I divided the atoll into seven geographical sectors: North, East, Southeast, South, Southwest, Northwest, and Deep (the central part of the lagoon), based on previous monitoring surveys of benthic cover, fishes, and invertebrates (Cover and McCully, unpublished data) and the atoll's geography (Figure 4). I used geographic information system (GIS) tools Hawth's Tools (Beyer 2009) and Geospatial Modelling Environment (Beyer

2011) to randomly select three to seven survey sites in each combination of habitat and sector.

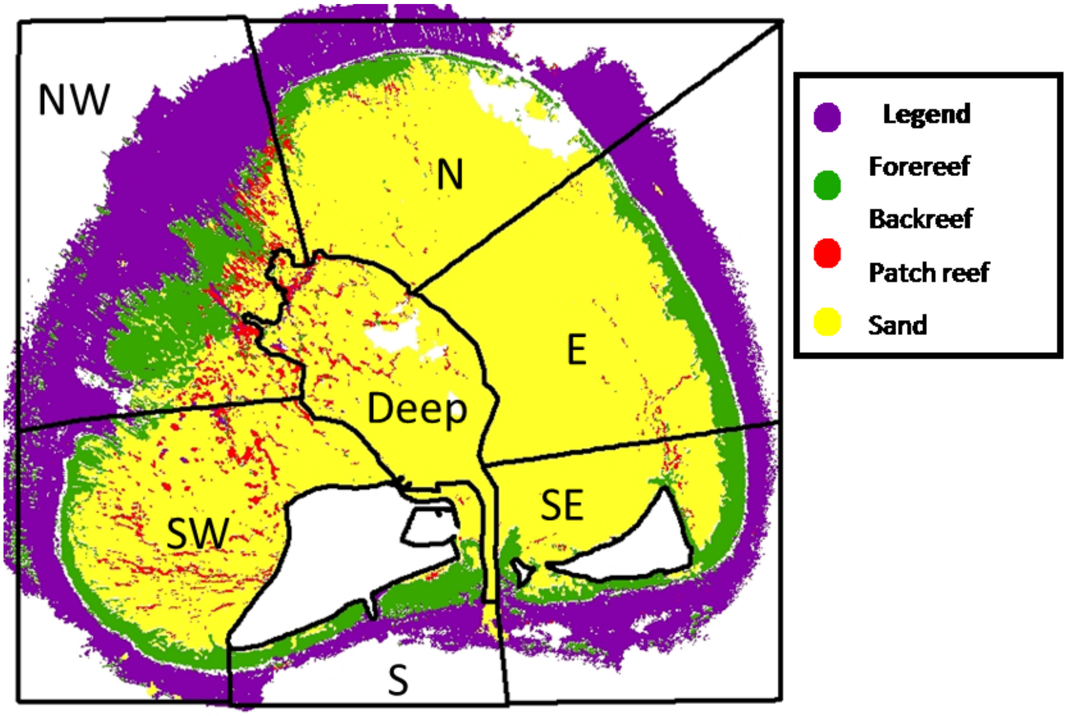

Figure 4: Habitats and geographical sectors of Midway Atoll, based on remote sensing classifications of NOAA (2003). White areas are excluded from surveys, as they are land, clouds in the satellite image used, exposed reef rim, deeper than 20 m, or the anthropogenic harbor.

Belt-Transect Methodology

At each pre-selected GPS location, I first checked that the site included the correct habitat. Due to limitations of NOAA (2003) classifications, a few Patch Reef sites had only sand, some Sand sites were situated over patch reefs, and a few chosen sites were too deep (>20 m deep) or too close to the reef rim for safety. In these cases, I went to the nearest correct habitat, generally within 100 m, and recorded the new GPS location.

At each site, I deployed a 25 m transect tape either towards and perpendicular to the closest reef rim for Backreef and Forereef sites, along the longest axis of the patch reef at Patch Reef sites, or to the east for Sand sites. Two divers counted, identified, and measured with calipers the largest dimension of every bivalve on hard substrate within 2 m of either side of the transect tape (for a total area of 100 m^2). Any individual that could not be identified in the field was photographed for later identification. I also recorded maximum and minimum depth of the survey area and brief observations of benthic cover, invertebrate and fish diversity, rugosity, and wave energy and took a variety of photographs.

At Sand sites, divers were generally able to examine the underside of each piece of rubble and thus often found more small bivalves (≤ 5 cm); at other sites, divers randomly examined the underside of a random sampling of rubble. Divers spent about an hour on each survey, using SCUBA for sites deeper than 5 m for Sand sites (which had little hard substrate to host bivalves) or >1 m for other habitats.

These surveys studied only epifaunal bivalves because studying infaunal bivalves requires collecting sediment cores and processing bivalves on land (Jones et al. 1990). I am unaware of any studies of infaunal bivalves in the Northwestern Hawaiian Islands.

I conducted 60 surveys in summer 2009 and 48 in summer 2011, for a total of 108 surveys (Figure 5). I conducted only three surveys in the Sand habitat in each sector because the focus was on large bivalves associated with hard substrate. Such bivalves were very rare at Sand sites (occasionally on rubble). I included Sand surveys mainly for ground-truthing the remote sensing classification of Sand habitat in NOAA (2003). I conducted three to seven surveys in all other habitats in each sector. Backreef and Forereef habitats do not exist in the Deep sector because this sector is in the middle of the lagoon and does not contain any atoll rim defining these habitats.

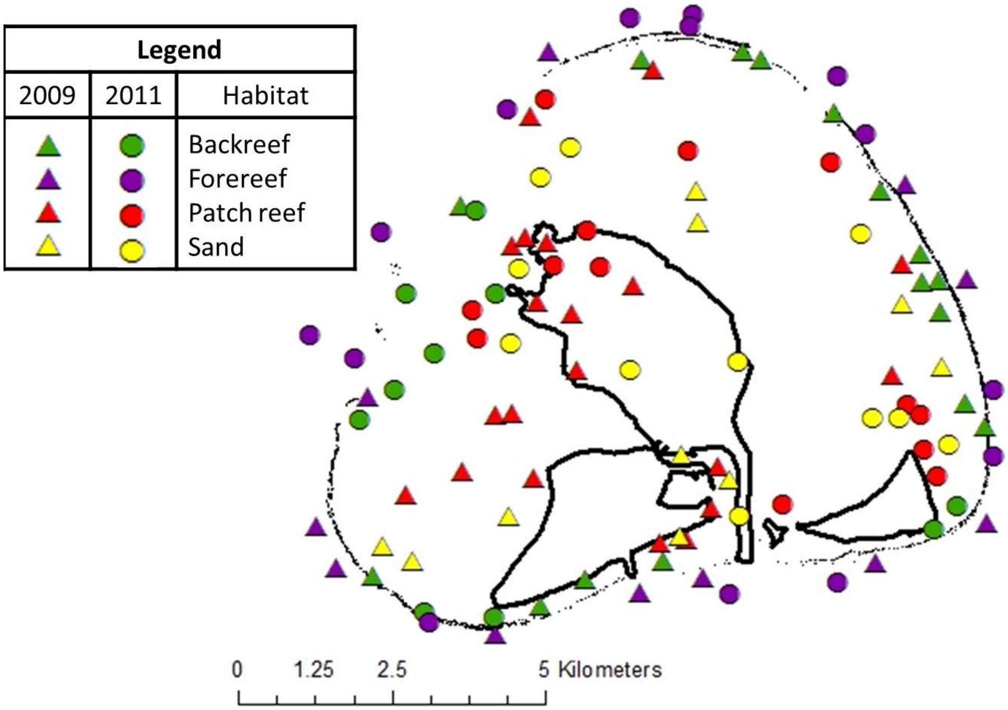

Figure 5: Sites of bivalve surveys. Triangles indicate surveys conducted in 2009 and circles those conducted in 2011. Colors indicate habitat: green – backreef, purple – forereef, red – patch reef, and yellow – sand. Black lines outline the atoll reef rim, islands, and the deep area in the center of atoll.

Data Analysis

To determine how habitat and spatial clustering contribute to bivalve distribution, I used a generalized linear model (GLM) on bivalve abundance with the factors Habitat and Sector and the interaction of Habitat and Sector. Using the programming language R (R Core Team 2012), I determined the best probability distribution for each species using Akaike's Information Criterion (AIC) and Chi-Squared Goodness-of-Fit (GOF) test and determined if there were significant differences among the habitats using a GLM with the best probability distribution. I determined which habitats were different from each other using Tukey's HSD posthoc test. To check for spatial structure not accounted for by the model, I then conducted a spatial autocorrelation analysis (Moran's I) on deviance residuals from the GLM using ArcMap (ESRI 2012).

I compared size distributions for the three common bivalves species in the three habitats that contained them with a standard one-factor ANOVA, assuming normal distributions, followed by Tukey's HSD posthoc tests to determine which habitats differed from others.

I estimated the total Midway population sizes of the three most common bivalve species using standard formulae for estimating population size (y_{str}) and standard error (s_{ystr}) from stratified sampling (Quinn and Keough (2002):

$$\bar{y}_{str} = \sum_{h=1}^{l} W_h \bar{y}_h \qquad \text{and} \qquad s_{\bar{y}_{str}} = \sqrt{\sum_{h=1}^{l} (W_h)^2 \frac{s_h^2}{n_h}} \quad ,$$

where l is the number of strata, W_h is the number of transect units (100 m^2) in stratum h (area/100), y_h is the sample mean for stratum h, s_h^2 is the sample variance for stratum h, and n_h is the number of surveys within stratum h (Quinn and Keough 2002: 156). I determined the area of each stratum by determining the number of 4-m x 4-m pixels in each stratum in the remote sensing classification. I estimated population size and standard error for each species using the 28 combinations of 7 geographic sectors and 4 habitats as strata.

The bivalve clearance time of a body of water is the "time that is theoretically needed for the total bivalve filter feeder biomass within an ecosystem to filter particles from a volume of water equivalent to the total system volume" (Smaal and Prins 1993, in Dame and Prins 1998). I calculated the bivalve clearance time for Midway Atoll's lagoon by using the median size of individuals in each population to estimate individual clearance rates using published equations and data for each species (or its closest relative for which data are available). Dame and Prins (1998) provide an equation for bivalve clearance rate (τ_f):

$$\tau_f = \frac{h}{F\ B} \text{x} \frac{10^3}{24}$$

where h = water depth (m), F= weight-specific filtration rate for a single benthic animal (l h^{-1} g^{-1}), and B= total weight of benthic filter-feeding population (g m^{-2}).

I modified the equation to include the total volume of the lagoon V(m^3) and the sum of the products of filtration rate of a median-sized individual (F_i, l h^{-1}), and number of individuals (N) for each species (S):

$$\tau_f = \frac{V}{\sum_i^S F_i N_i} \text{x} \frac{10^3}{24}$$

Using remote sensing bathymetry data in NOAA (2003), I estimated the volume of Midway's lagoon by calculating the sum of the products of the number of pixels in the lagoon and their estimated depths.

Results:

The three most common bivalve species were: cliff oysters *Spondylus violacescens* (n=144), jewelbox oysters *Chama limbula* (n=76), and baggy pen shells *Streptopinna saccata* (n=90) (Figure 6). *C. limbula* was previously known in Hawaii as *Chama iostoma* Conrad, 1837 (Rosenberg 2013). I also observed 11 ventricose arks *Arca ventricosa*, 3 scallops (family Pectinidae), 5 *Pinctada margaritifera* (mostly on a patch reef in the central Deep sector in 2009), 2 small *Pinctada* spp. (likely *P. maculata* as in McCully, unpublished), one *Isognomon incisum*, and 25 bivalves that could not be identified in field or by photograph. Taxonomy of these bivalves is provided in Table 2.

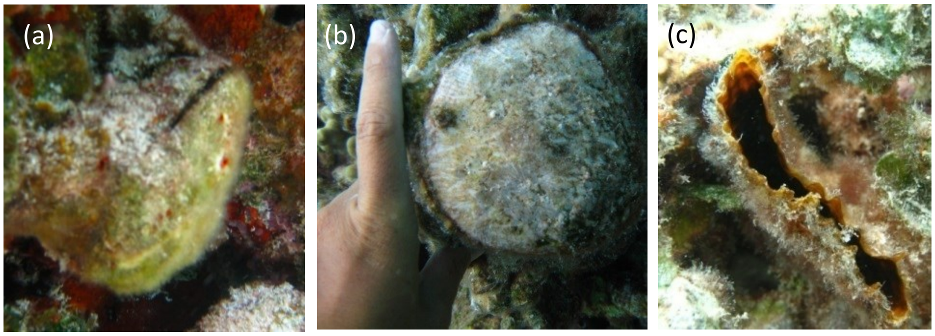

Figure 6: Three most common bivalves observed at Midway Atoll are: (a) *Spondylus violacescens*, (b) *Chama limbula*, and (c) *Streptopinna saccata*.

Table 2: Taxonomy of all epifaunal bivalves (Phylum Mollusca, Class Bivalvia) observed on Midway Atoll during this study. Taxonomic source: World Register of Marine Species (http://www.marinespecies.org/). Species used in statistical analysis are in bold.

Subclass	Pteriomorpha	
Order	Arcoida	
Superfamily	Arcoidea	
Family	Arcidae	*Arca ventricosa* Lamarck 1819 Ventricose ark
Order	Pectinoida	
Superfamily	Pectinoidea	
Family	Spondylidae	***Spondylus violacescens* Lamarck 1819** **Cliff oyster**
Superfamily	Pectinoidea	
Family	Pectinidae	Various scallop species
Order	Pteroida	
Superfamily	Pinnoidea	
Family	Pinnidae	***Streptopinna saccata* (Lamarck 1758)** **Baggy pen shell**
Superfamily	Pteroidea	
Family	Pteriidae	***Pinctada margaritifera* (Linnaeus 1758)** **Black-lipped pearl oyster** *Pinctada maculata* (Gould 1850) Pipi pearl oyster *Isognomon incisum* (Conrad 1837) Incised purse shell
Subclass	Heterodonta	
Order	Veneroida	
Superfamily	Chamoidea	
Family	Chamidae	**Chama limbula Lamarck 1819** **Jewelbox oyster**

I conducted statistical analyses on only the three most common species (*S. violacescens*, *C. limbula*, and *S. saccata*) because these were the only species with more than 15 individuals across all 108 surveys. Because none of these species were present in any of the Sand surveys, I excluded Sand surveys from statistical analysis.

Spatial Distribution

Comparing six probability distributions with AIC and Chi-Squared goodness-of-fit (GOF) tests indicated that the negative binomial distribution was the best-fitting probability distributions for all three species, because this distribution had the lowest AIC value and the only GOF p-value <0.05. This is expected because the negative binomial distribution is commonly used to represent skewed distributions of counts of organisms (White and Bennetts 1996, Quinn and Keough 2002: 12).

I used R to run a separate GLM for the abundance of each species, assuming negative binomial distributions with Habitat (Backreef, Forereef, Patchreef) and Sector (seven sectors) as factors, and including the interaction between Habitat and Sector. These were followed by Tukey's HSD posthoc tests.

S. violacescens was significantly more abundant on the forereef than in the other two habitats, and it was more abundant on the East Forereef than in all other combinations of habitat and sector (Figure 7, Table 3).

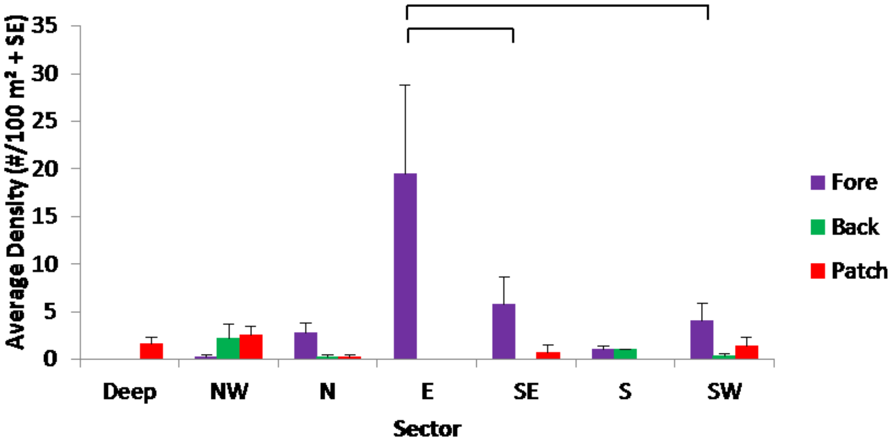

Figure 7: Abundance of *Spondylus violacescens* at Midway Atoll by Habitat and Sector. Lines at top indicate combinations of habitat and sector that are not statistically significant different from East Forereef (Tukey's HSD, p > 0.05).

Table 3: Results of (a) GLM and (b) Tukey's HSD tests comparing abundances of *Spondylus violacescens.* Tukey's HSD results with p > 0.15 are not shown. (*** = p<0.001, ** = p<0.01, * = p<0.05)

(a) Factor	Df	Deviance	Resid. Df	Resid. Dev.	P	
NULL			83	210.00		
Habitat	2	64.06	81	145.94	<0.001	***
Sector	6	21.75	75	124.19	0.001342	**
Habitat x Sector	10	58.51	65	65.68	<0.001	***

(b) Hypothesis	Estimate	Std. Error	z value	P	
Fore.E - Patch.Deep = 0	2.52	0.57	4.408	<0.01	***
Fore.E - Back.SW = 0	3.89	0.88	4.435	<0.01	***
Fore.E - Fore.S = 0	2.97	0.74	4.019	<0.01	**
Fore. E - Patch.SE = 0	3.26	0.79	4.106	<0.01	**
Fore.E - Patch.SW = 0	2.63	0.64	4.11	<0.01	**
Fore.E - Patch.N = 0	4.36	1.14	3.827	0.0104	*
Fore.E - Fore.NW = 0	4.36	1.14	3.827	0.0106	*
Fore.E - Back.N = 0	4.36	1.14	3.827	0.0107	*
Fore.E - Back.NW = 0	2.18	0.60	3.643	0.02	*
Fore.E - Back.S = 0	2.97	0.82	3.611	0.0222	*
Fore.E - Patch.NW = 0	2.02	0.59	3.431	0.0433	*
Fore.E - Fore.N = 0	1.94	0.58	3.33	0.0588	
∑Fore – ∑Back = 0	2.12	0.47	4.474	<0.0001	***
∑Fore - ∑Patch = 0	1.63	0.42	3.88	0.000288	***
∑E - ∑S = 0	2.24	0.77	2.91	0.055	

Although there were statistically significant differences for Sector and the interaction between Sector and Habitat in the GLM for *C. limbula*, the Tukey's HSD posthoc test found no statistically significant difference between pairs of Habitats, Sectors, or combinations of Habitat and Sector (Table 4). Figure 8 shows that *C. limbula* were most abundant on the forereef in most of the sectors, but also abundant on patch and back reef in the Deep, NW, and SW sectors. The non-significant posthoc test despite the significant F-test suggests that there is insufficient statistical power to resolve the posthoc comparisons, perhaps due to small sample sizes and/or large numbers of groups.

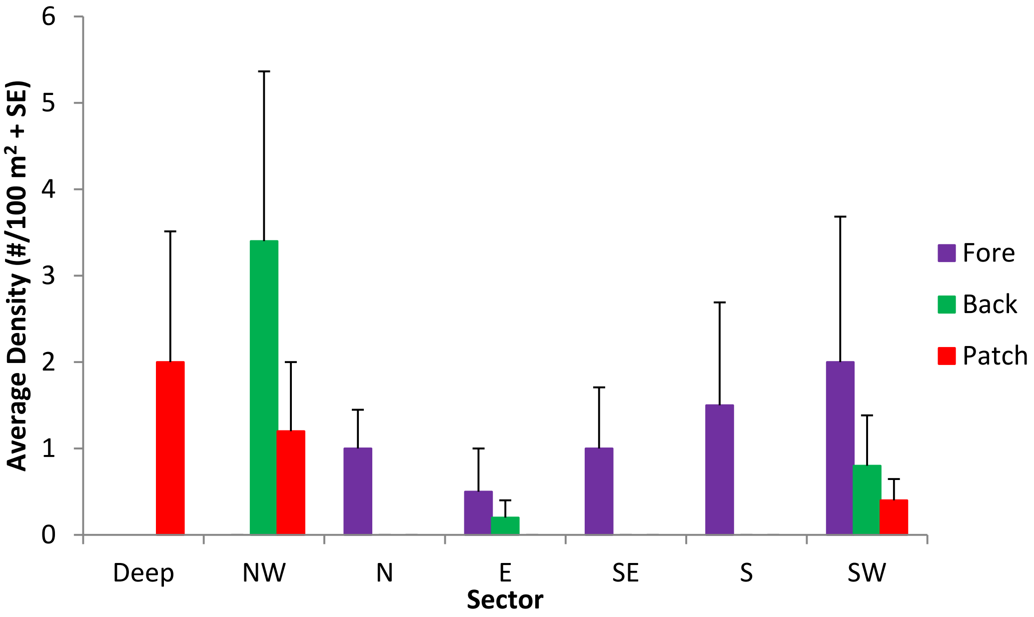

Figure 8: Abundance of *Chama limbula* at Midway Atoll by Habitat and Sector.

Table 4: GLM analysis of abundance of *C. limbula* (*** = p<0.001, ** = p<0.01, * = p<0.05).

Factor	Df	Deviance	Resid. Df	Resid. Dev.	P	
NULL			83	97.49		
Habitat	2	0.89	81	96.61	0.64	
Sector	6	26.79	75	69.82	0.0001588	***
Habitat x Sector	10	22.79	65	47.69	0.0144402	*

S. streptopinna was significantly more abundant on the forereef than in the other two habitats (Figure 9, Table 5). Over 80% of *S. streptopinna* seen were on the forereef. *S. saccata* were over 10 times as abundant on the forereef as on the other two habitats.

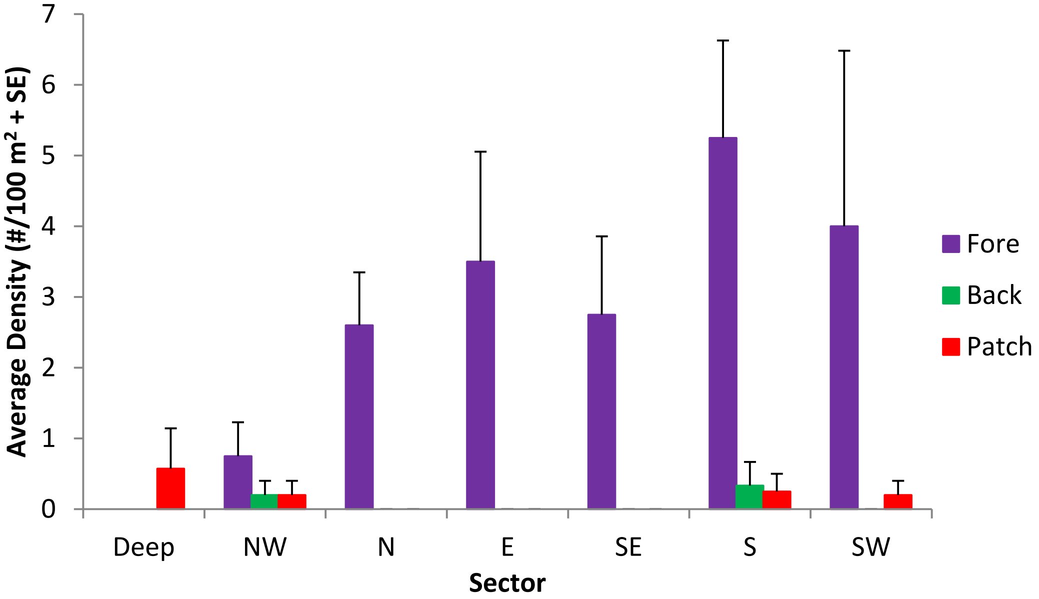

Figure 9: Abundance of *Streptopinna saccata* at Midway Atoll by Habitat and Sector.

Table 5: (a) GLM and (b) Tukey's HSD results for abundance of *S. streptopinna* (*** = p<0.001, ** = p<0.01, * = p<0.05)

(a) Factor	Df	Deviance	Resid. Df	Resid. Dev.	P
NULL			83	168.58	
Habitat	2	97.07	81	71.51	<2 x 10^{-16} ***
Sector	6	10.47	75	61.04	0.1063
Habitat x Sector	10	9.76	65	51.29	0.4620

(b) Hypothesis	Estimate	Std. Error	z value	P	
Fore – Back = 0	3.7028	0.7588	4.880	< 1 x 10^{-5}	***
Fore – Patch = 0	2.6884	0.4605	5.838	< 1 x 10^{-5}	***
Patch - Back = 0	1.0144	0.8355	1.214	0.432	

Deviance residuals for the three species were not spatially autocorrelated by inverse distance, nearest neighbors (4 and 8), or fixed distance (500m,1000m, and 2000m) algorithms. This suggests that the factors used in the GLMs for each species adequately accounted for the spatial structure.

Size Frequency Distributions

The size distributions of *S. violacescens* varied significantly among the three habitat types (Tables 6 and 7). Figures 10 and 12a show that individuals on the forereef were very large (most greater than 100 mm), while individuals in the backreef were very small (most smaller than 30 mm) and individuals on patch reefs were more evenly distributed with an intermediate mean size.

C. limbula individuals in the back reef were significantly smaller than those on patch reefs and the forereef (Tables 6 and 7, Figure 10). Figure 12c shows that over half of the individuals observed on the back reef were less than 20 mm, while only a few individuals in the other habitats were this small. Around half of the individuals on patch reefs and the forereef were greater than 80 mm.

There was no significant difference in the sizes of *S. saccata* individuals among the three habitats, but this is likely because less than 10 individuals were observed on either patch reefs or the backreef (Tables 6, Figures 10 and 12c).

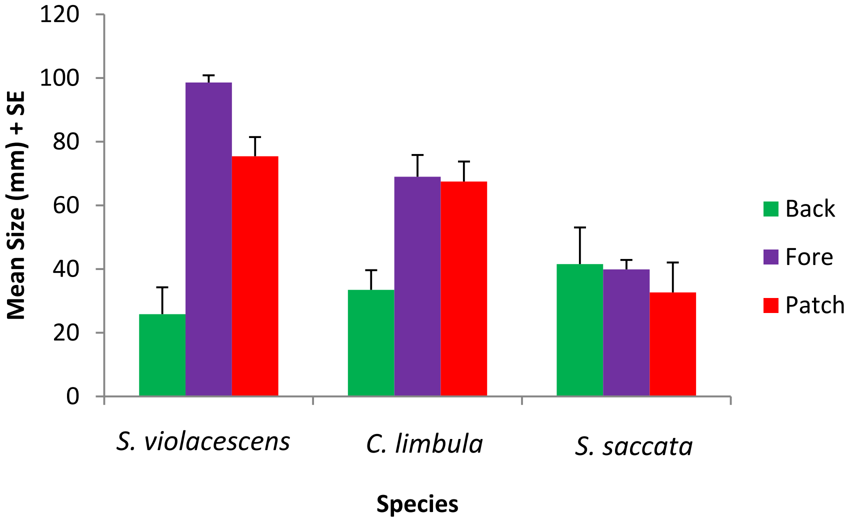

Figure 10: Mean and standard error of size of 3 species in Backreef, Forereef, and Patchreef habitats.

Table 6: ANOVA analysis for size of (a) *S. violacescens*, (b) *C. limbula,* and (c) *S. saccata* (*** = <0.001, ** = <0.01, * = <0.05)

Factor	Df	SS	MS	F	P
(a) *Spondylus violacescens*					
Habitat	2	94062	47031	54.55	< 0.001 ***
Residuals	187	161231	862		
(b) *Chama limbula*					
Habitat	2	21818	10909	10.39	< 0.001 ***
Residuals	74	77670	1050		
(c) *Streptopinna saccata*					
Habitat	2	359	179.4	0.262	0.77 ns
Residuals	87	59456	683.4		

Table 7: Tukey's HSD Posthoc analysis contrasting individual size between habitats

	S. violacescens	***C. limbula***
Back vs Fore	< 0.001 ***	< 0.001 ***
Back vs Patch	< 0.001 ***	0.001 **
Fore vs Patch	< 0.001 ***	0.986 ns

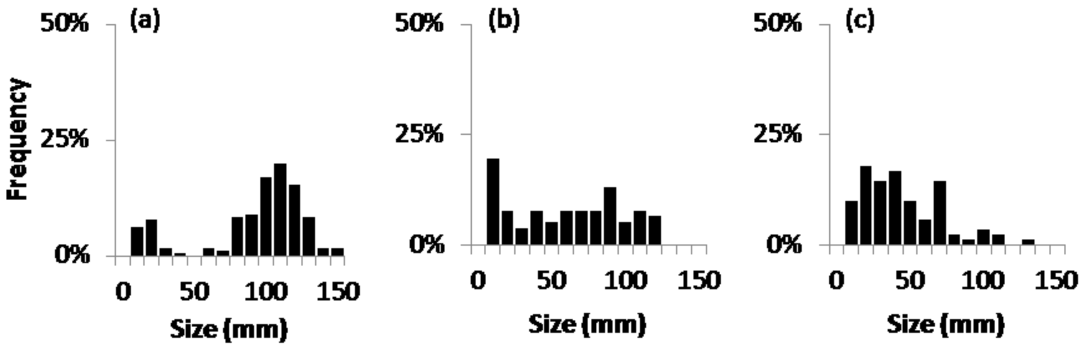

Figure 11: Size frequency distribution across all sites of (a) *Spondylus violacescens*, (b) *Chama limbula*, and (c) *Streptopinna saccata*.

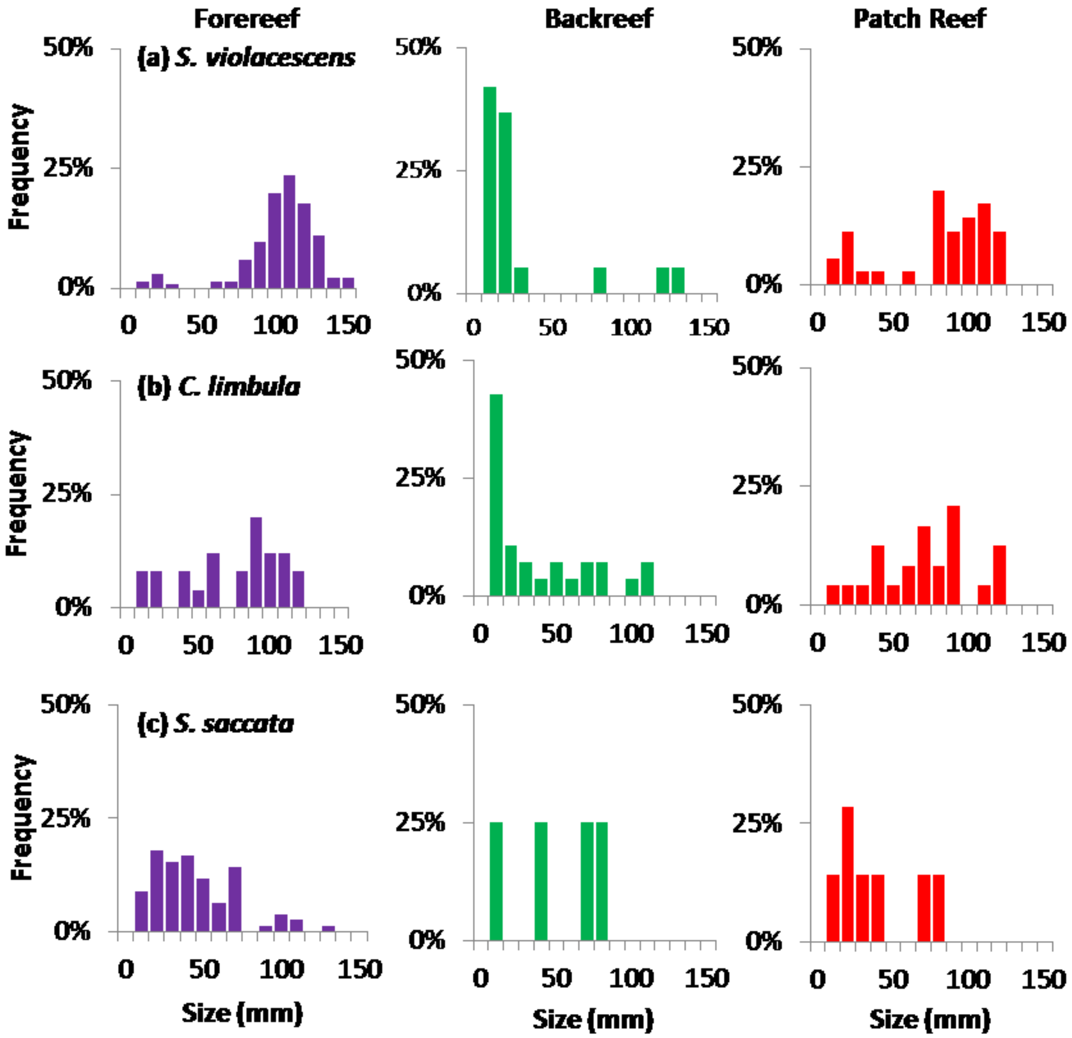

Figure 12: Habitat-specific size-frequency distributions for (a) *Spondylus violacescens*, (b) *Chama limbula*, and (c) *Streptopinna saccata.*

Total Population Abundance

Stratified surveys allow one to estimate total population abundances, taking account of varying densities among habitats and sectors (Figure 13). *S. violacescens* is the most common bivalve at Midway Atoll, with a total population of 1,272,815 ± 326,079 individuals across all parts of Midway Atoll; *S. saccata* is next with a total population of 630,813 ± 119,650 individuals; and *C. limbula* is the least common bivalve, with a total population of 347,594 ± 97,924 individuals.

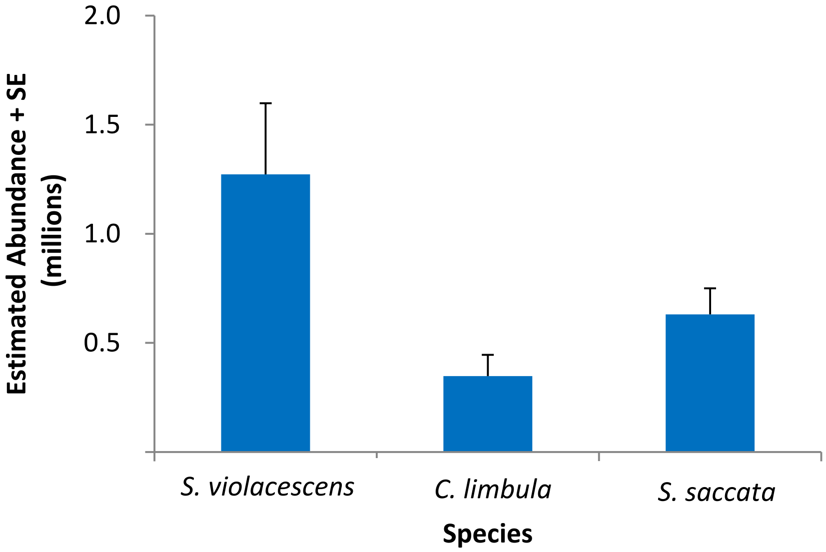

Figure 13: Estimated abundance of bivalve species using 28 combinations of 7 sectors and 4 habitats.

Bivalve Clearance Time

To estimate individual clearance rates for median-sized *C. limbula*, *S. violacescens*, and *S. saccata* at Midway Atoll, I used published clearance rate equations for *Chama iostoma* (a junior synonym of *C. limbula*), for *Chlamys hysta*ta, a temperate Pacific North American species in a sister family to the Spondylidae, and *Atrina tuberculosa*, a tropical Pacific North American species in a sister genus to *Streptopinna* (Table 11).

Table 11: Published formulae used for calculating individual clearance rates for median-sized bivalve species at Midway Atoll. F_i = individual clearance rate (L h^{-1}); W = tissue dry weight (g); and H = shell height (mm).

Source species	Equation	Reference
Chama iostoma	$F_i=7.63\ W^{0.71}$	Addessi (1999) in Niquil et al. (2001)
	$H = 35.96 * \log(W) + 32.61$	Richard (1985)
Chlamys hystata	$F_i=8.71\ W^{0.94}$	Meyhofer (1985, in Pouvreau et al. 1999)
	$W=0.0022H^2-0.1055H+1.3387$	
		Calculated from MacDonald et al. (1991)
Atrina tuberculosa	$F_i=1.177$ l h^{-1} g^{-1}	Nieves-Soto et al. (2013)

Table 12: Calculations of estimated population clearance rate within the Midway Atoll lagoon. Median dry weight of *S. saccata* is a very rough estimate since no information was available in the literature on the relationship between length and dry weight.

Species	Median Size (mm)	Median Dry Weight (g)	Population Size	Clearance Rates (L h^{-1})	
				Individual	Population
S. violacescens	99.3 (n=190)	12.56	153,062	93.96	144×10^5
C. limbula	56.9 (n= 77)	4.73	162,314	23.02	37.4×10^5
S. saccata	32.9 (n= 90)	5.00	19,217	5.885	1.13×10^5
All species					182.31×10^5

An analysis of NOAA's (2003) bathymetric atlas resulted in an estimate of 214.5 million m^3 of water inside Midway's lagoon, very similar to the estimate of 213 million m^3 by Hoeke et al. (2006). After deducting the part of each species' population (>50%) estimated to live on the forereef, the remaining population sizes inside the lagoon in Table 11 are much smaller than the total population sizes in Figure 13. The combined filtration rate for all three populations is estimated to be 18.2 million L h^{-1}. *S. violacescens* contributes 79% of the total filtration capacity due to the large size and high filtration rates of typical individuals and its abundance. The combined clearance time for the Midway lagoon by these three species is estimated to be about 490 days.

Discussion:

Spatial and Size Distributions of Bivalves

Two of the three species were most common on the forereef. Overall, *S. violacescens* was significantly more abundant on the forereef than in the other habitats and especially on the east forereef where it was more abundant than nearly all other combinations of habitat and sector. While *C. limbula* was most abundant on the forereef in most sectors, it was also abundant on patch and back reefs in the deep central, northwestern, and southwestern sectors. However, Tukey's HSD posthoc tests found no significant differences between pairs of habitats, sectors, or combinations of habitats and sectors. The distribution of *S. saccata* is restricted primarily by habitat, with nearly all individuals found on the forereef. *S. violacescens* individuals were largest on the forereef and smallest in the backreef. *C. limbula* individuals were much smaller in the backreef than individuals on the forereef and on patch reefs. These observations are consistent with observations made on other Indo-Pacific reefs and may be explained by the life histories of these species.

All three species are suspension-feeding bivalves with larval stage that attach permanently to hard substrate. All three initially attach with byssal threads (e.g., *S. saccata*, Seilacher 1984, Zuschin et al. 2001) or are likely to do so because congeners do (*Chama japonica*, Scarpa and Wada 1994; *Spondylus americanus*, Logan 1974). Although *S. saccata* generally settles in crevices of live or dead coral heads with only its outer edge exposed to predators, both *C. limbula* and *S. violacescens* cement their lower valves to hard substrates and are potentially very susceptible to predators (Figure 6, Zuschin et al. 2001, Zuschin et al. 2000).

S. saccata's almost complete limitation to the forereef is consistent with other observations of *S. saccata* on outer-reef slopes, at greater depths, and embedded in massive coral colonies. Paulay (1990) found *S. saccata* only on outer-reef slopes and one of three inner-reef habitats in presence/absence surveys of many South Pacific islands. Kay (1979) observed "abundant" Pinnidae at depths of 50 m off Necker, Nihoa and other NWHI. It is possible that *S. saccata* is primarily limited to deeper habitats, since the forereef reaches much greater depths (all sites > 5 m) than patch reefs (2-8 m) and back reef (1-6 m, Table 1). As well, Zuschin et al. (2000) reported that *S. saccata*, in the northern Red Sea, is usually embedded in living massive coral colonies or sometimes in crevices of dead coral heads. These massive coral colonies (primarily *Porites lobata* and *P. evermanni* at Midway) are more common on the forereef than on patch reefs and most back reef habitats (Table 1).

The great abundance of *S. violacescens* on the forereef may be explained by the high frequency of crevices and ledges on the forereef (Table 1), which provide refuge microhabitats from predators (Feifarek 1987). Potential predators of epifaunal bivalves include gastropods, spiny lobsters, rays, porcupinefish and other large fishes, stomatopods, and sea turtles (Feiferak 1987, Stone 1998). Many authors speculate that the adaptive value of the spines of *Spondylus* is to directly deter predators or host encrusting algae and invertebrates that camouflage against predators (Feiferak 1987, Logan 1974, Stone 1998, and others). Feifarek (1987) showed experimentally that predation on *S. americanus* was lower for larger individuals, those next to or under ledges, those in deeper water, and those with neither spines nor epibionts removed. The particularly high abundance of *S. violacescens* on the eastern forereef may be related to the high wave energy hitting this region for much of the year driven by northeasterly tradewinds, which may create more crevices and ledges on the eastern side of the forereef than in other regions of the forereef.

S. violacescens and *C. limbula* individuals were larger on the forereef than in the other habitats, perhaps because larger individuals are less vulnerable to predation than smaller individuals (Feifarek 1987) and because predators on small individuals may be more common on the forereef than in the other habitats.

These explanations for the observed abundance and size distributions are only hypotheses based on my and others' observations of these species and the habitats and need to be explored experimentally before being accepted.

Total Population Abundance

This study shows that *S. violacescens* is the most common bivalve at Midway Atoll, with a total population around 1.3 million individuals. *C. limbula* is the least common bivalve, with a total population of about 0.35 million individuals, while *S. saccata* has around 0.63 million individuals. Thus, the total number of these bivalves at Midway Atoll is estimated to be 2.25 million individuals.

Similar surveys quantifying abundances and distributions of bivalves in coral reef habitats are relatively rare (Zuschin et al. 2001). These surveys usually focus on one species (e.g., Zanini and Salvat 2000), only on soft-substrate bivalves (e.g., Jones et al. 2006, Weber and Zuschin 2013), have relatively few stations (e.g., Zuschin and Piller 1997). Other studies only record presence/absence of bivalve species as part of a search for introduced species (e.g., Coles et al. 1999), comparison of mollusk diversity to past surveys (e.g., Augustin et al. 1999), or as a first effort at surveying an area (e.g., Adjeroud and Salvat 1996). An ISI Web of Science search for “coral reef bivalve abundance” returned 93 records, but only four were attempts to quantify abundance and distribution of bivalves.

Zuschin et al. (2001) estimated densities for a variety of hard-substrate mollusks in different habitats of the Bay of Safaga in the northern Red Sea coast of Egypt. Although they also observed individuals in the taxa Chamoidea, Spondylidae, and *Streptopinna saccata*, the most common mollusks were either not known from Midway Atoll (e.g., *Pedum spondyloideum*), were not present in Hawaii (e.g., *Tridacna maxima*), or were gastropods not included in this study. Of the genera observed in both studies, Chamoidea (mostly *Chama brassica* and *imbricata*) were the most common in nearly all habitats, with highest densities in rock grounds (0.5 per 0.25 m^2) and faviid carpets (0.1 per 0.25 m^2). 47 of 50 observed Spondylidae (mainly *Spondylus marisrubi*) were on faviid carpets (<0.1 per 0.25 m^2). Nearly all *Streptopinna saccata* were embedded in massive *Porites* colonies (0.1 per 0.25 m^2) or on faviid carpets (0.0 per 0.25 m^2). Rock grounds were primarily exposed coral limestone with some scleractinian and soft corals, while faviid carpets included a coral framework with variable-height coral colonies providing diverse molluscan habitats. Both these habitats had low light levels and low coral cover, perhaps because of high sediment and organic material load. It is difficult to compare habitat associations across studies because the two studies did not use the same habitats and the two locations were extremely different (a deep bay and a shallow atoll), but in both studies *Spondylus* and *Chama* were most dense in areas with relatively low coral cover (which at Midway includes almost all areas except the northeast back reef). Although *S. saccata* is described by Zuschin et al. (2001) as living in crevices of living corals, we have not observed this at Midway, perhaps because living corals are relatively rare. It is also interesting that *Spondylus* was almost completely restricted to one habitat in the Red Sea study, but was in all habitats and sectors on Midway, although concentrated primarily on the forereef and northwestern backreef and patch reefs. Because Zuschin et al. (2001) do not report area estimates of each of their habitats, they were not able to estimate a total population size for each species. However, in their most dense habitats, all three of these taxa were an order of magnitude denser in the northern Red Sea than at Midway Atoll.

Taylor (1984) quantified the abundance and diversity of bivalves and gastropods at two offshore patch reef sites and two fringing reef sites in the Sudanese Red Sea. He found a much greater density and species richness of bivalves at the patch reef sites than at the fringing reef sites. He also found a much greater abundance (0.2-3.8 bivalves/m^2) and species richness (3-28 species) in a much smaller area (16-32 m^2) than I found at Midway. This discrepancy may result from closer and slower examination of a smaller area in Sudan than at Midway, where I surveyed much larger areas and subsequently focused on larger individuals.

Salvat conducted numerous studies of the molluscan fauna of French Polynesian reefs, including reefs in the Austral Islands, Gambier Archipelago, and Tuamotu Archipelago. Salvat (1971) compares mollusks of Raevavae Island (Austral Islands) to islands in the Gambier and Tuamotu archipelagoes, finding densities and biomass of the same order of magnitude at all three reefs (~5 individuals/ m^2 and 1 g/m^2). Although these densities are higher than we observed at Midway, the biomass suggests that most of these individuals are very small and these numbers also included gastropods. The five species that dominated both numerical and biomass estimates at Raevavae were all gastropods, so it is impossible to generate estimates for bivalves to compare with Midway. Many of Salvat's other studies only report presence/absence data to document biodiversity (e.g., Salvat and Erhardt 1970, Adjeroud and Salvat 1996).

Niquil et al. (2001) used total population estimates of farmed and natural bivalve stocks to estimate bivalve consumption of planktonic primary production in the lagoon of Takapoto Atoll, Tuamotu Archipelago, French Polynesia. Although the methods are not readily available, all these studies probably used methods similar to those used in Zanini and Salvat (2000) to estimate natural *P. margaritifera* stocks: they combined estimates of density of bivalves in bottom type and bathymetric strata obtained from scuba censuses and estimates of area of bottom type in bathymetric strata obtained from depth-sounding profiles. These methods are relatively similar to those I used at Midway, except that I didn’t use specific bathymetric strata (since 98% of area inside the lagoon falls within the first stratum of 0-10 m and all forereef surveys fell within the second stratum of 10-20 m) and I used remote sensing to estimate the area of each stratum rather than depth-sounding profiles. However, Zanini and Salvat (2000) surveyed only inside the lagoon of the closed and deep atoll to a depth of 50 m, while we surveyed both inside and outside the atoll to a depth of 20 m. The two atolls are about the same size (Table 8), but Takapoto’s lagoon is much deeper and is completely enclosed by a reef rim, except for a few channels <50 cm deep. Pearl oysters (*P. margaritifera* and *P. maculata*) and *Arca ventricosa* are at least three orders of magnitude less dense at Midway than at Takapoto. *C. limbula* (called *C. iostoma* in Niquil et al. 2001) are about two orders of magnitude less dense at Midway Atoll than at Takapoto Atoll. The most common bivalve at Midway Atoll, *S. violacescens*, was not included in the Takapoto study, although it is reported in French Polynesia (GBIF 2013).

Table 8: Comparisons of physical characteristics and bivalve stocks of Midway Atoll and Takapoto Atoll. Population size of bivalves at Takapoto Atoll includes only bivalves inside lagoon, while population size of bivalves at Midway Atoll includes bivalves inside the lagoon and outside the atoll to 20 m depth.

	Midway Atoll (this study)		Takapoto Lagoon (Niquil et al. 2001)	
Geographic location	Northwestern Hawaiian Islands		Tuamotu Archipelago, French Polynesia	
Total reef area	348 sq km (89.2 sq km included in surveys)			
Total reef area in lagoon	70 sq km		81 sq km	
Mean depth	3 m		25 m	
Population size of natural bivalve species (excluding cultured populations)	*S. violacescens*	1.3×10^6	*Arca ventricosa*	635×10^6
	S. saccata	0.63×10^6	*C. iostoma/ limbula*	53×10^6
	C. limbula	0.35×10^6	*Pinctada maculata*	31×10^6
			P. margaritifera	4×10^6
Bivalve clearance time	~490 days		73 days	
Water residence time	< 7.5 days		1,460 days	

Bivalve Clearance Time

Bivalve clearance time for Midway's lagoon is estimated to be about 490 days. By comparing this figure to Takapoto Atoll Lagoon (Table 13, Niquil et al. 2001) and 11 other marine ecosystems (Dame and Prins 1998), we can see that this is a long clearance time. Midway's bivalve clearance time is second only to that of Delaware Bay (1278 days, where bivalve populations were historically much larger) and much higher than Takapoto Lagoon (73 days). Midway's bivalve clearance time is similar to bivalve clearance times of Carlingford Lough (490.2 days) in Ireland where bivalve culture is just beginning to impact a pelagic system and Chesapeake Bay (325 days) where bivalve populations were historically much larger. Since Midway's lagoon is quite shallow compared to Takapoto Atoll and most of the other marine ecosystems, this comparison shows that that the filtration capacity of Midway's bivalves is quite low.

Water flow into an atoll includes ocean currents, tidal exchange, rainfall, and waves and can vary from season-to-season or year-to-year (Keenan 2007). Bivalve clearance time is often compared to a measure of water flow called water residence time, which is the theoretical time it takes for the volume of water within a basin to be replaced with water from outside the system (Dame and Prins 1998). Although no estimate of water residence time in Midway's lagoon is available, its volume is an order of magnitude smaller than that of Pearl and Hermes Atoll and both atolls have large openings on their west sides, so Midway's water residence time is likely less than Pearl and Hermes Atoll's estimated water residence time of 7.5 days (Hoeke, in prep, in Keenan 2007).

Dame (2012a) argues that, in systems with residence times less than bivalve clearance times, such as Midway, bivalves probably only influence plankton at the scale of the bivalve bed or community, rather than at the scale of the whole lagoon.

The total number of individuals at Midway Atoll is about two orders of magnitude smaller than the number of bivalves living naturally in the similarly-sized lagoon of Takapoto Atoll (Table 13), which is the only coral reef site for which a comparable study has estimated population sizes. Midway's bivalve clearance time (over a year) is about seven times that of Takapoto Lagoon, where Niquil et al. (2000) conclude that total consumption by farmed and benthic bivalves is a very small percentage of planktonic primary productivity (5.32%). Although an estimate of planktonic primary productivity at nearby French Frigate Shoals is less than half of the estimate for Takapoto Lagoon (Polovina 1984), this comparison to Takapoto Lagoon further supports the assertion that Midway's bivalves are very unlikely to limit Midway's planktonic primary productivity. This is consistent with Dame's (2012c) observation that bivalve-dominated ecosystems (e.g., oyster reefs) tend to be in temperate brackish water.

However, a more detailed model of the role of bivalve filtration at Midway should also account for the amount and rate of the production of organic carbon by phytoplankton and how effectively that carbon is removed by bivalves (Dame 2012a). Midway also contains many other filter-feeding organisms, such as corals, sponges, and tunicates that may further influence or control plankton.

Conclusions

Surveys of epifaunal bivalves at Midway Atoll identified three main bivalve species on hard substrates, with different patterns of distribution related to habitat and/or geographic location. Two of these species are significantly more common on the forereef than on patch reefs or the back reef. The total number of individuals of these three species at Midway Atoll is estimated to be about 2.25 million individuals. Although very few studies have quantified bivalve population size in coral reef habitats, the density of bivalves at Midway is much less than densities reported in Takapoto Atoll in French Polynesia (Niquil et al. 2001), the Sudanese Red Sea (Taylor 1984), and the Egyptian Red Sea (Zuschin et al. 2001). The number and size of bivalves inside the atoll's lagoon is likely insufficient to control plankton abundance and productivity inside the lagoon.

Further studies at Midway should include experiments examining the proposed mechanisms for the observed spatial distributions of bivalves, surveys of infaunal bivalves not included in these surveys, and better estimates and a more detailed model of bivalve filtration, plankton productivity, and water movement.

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Appendix

Table A1: Categorization of NOAA's (2003) 21 benthic habitats from remote sensing classification into the 4 benthic habitat types used in this study.

DN	NOAA Classification	Depth	Main Habitat
23	Hardbottom, coral colonized		Backreef
26	Hardbottom, uncolonized	<10 m	
160	Pavement		
163	Pavement, coral colonized		
165	Pavement, coral colonized + dense algae		
166	Pavement, uncolonized		
17	Groove		Forereef
26	Hardbottom, uncolonized	>20	
80	Spur and Groove		
161	Pavement + sparse algae		
162	Pavement + dense algae		
186	Pavement with sand channels, uncolonized		
11	Sand		Sand
230	Macroalgae on sand		
26	Hardbottom, uncolonized	<= 20 m & >= 10 m	Patch Reef
100	Patch Reef		
103	Patch Reef, coral colonized		
180	Pavement with sand channels		
183	Pavement with sand channels, coral colonized		
29	Hardbottom, crustose coralline algae		Reef Rim
40	Linear Reef		
242	Reef crest		
253	Surf		

Table A2: Area and abundance of bivalves in each combination of geographic sector and habitat, as used to estimate abundance. n_h = number of surveys

Sector	**Habitat**	**Area (m^2) (100 * W_h)**	n_h	***S. violacescens***		***C. limbula***		***S. saccata***	
				Mean (y_h)	**SD (s_h)**	**Mean (y_h)**	**SD (s_h)**	**Mean (y_h)**	**SD (s_h)**
Deep	Back	78,848	0						
Deep	Fore	87,024	0						
Deep	Patch	537,504	7	1.57	2.07	2.00	4.00	0.57	1.51
Deep	Sand	7,755,744	3	0.00	0.00	0.00	0.00	0.00	0.00
E	Back	1,442,480	5	0.00	0.00	0.20	0.45	0.00	0.00
E	Fore	3,165,728	4	19.50	18.48	0.50	1.00	3.50	3.11
E	Patch	97,872	4	0.00	0.00	0.00	0.00	0.00	0.00
E	Sand	12,557,248	3	0.00	0.00	0.00	0.00	0.00	0.00
N	Back	1,228,192	4	0.25	0.50	0.00	0.00	0.00	0.00
N	Fore	3,985,168	5	2.80	2.17	1.00	1.00	2.60	1.67
N	Patch	191,232	4	0.25	0.50	0.00	0.00	0.00	0.00
N	Sand	10,071,424	3	0.00	0.00	0.00	0.00	0.00	0.00
NW	Back	3,446,928	5	2.20	3.35	3.40	4.39	0.20	0.45
NW	Fore	9,330,608	4	0.25	0.50	0.00	0.00	0.75	0.96
NW	Patch	1,069,760	5	2.60	2.07	1.20	1.79	0.20	0.45
NW	Sand	2,664,768	3	0.00	0.00	0.00	0.00	0.00	0.00
S	Back	1,459,296	3	1.00	0.00	0.00	0.00	0.33	0.58
S	Fore	1,748,560	4	1.00	0.82	1.50	2.38	5.25	2.75
S	Patch	44,464	4	0.00	0.00	0.00	0.00	0.25	0.50
S	Sand	823,680	3	0.00	0.00	0.00	0.00	0.00	0.00
SE	Back	1,950,592	4	0.00	0.00	0.00	0.00	0.00	0.00
SE	Fore	3,821,248	4	5.75	5.74	1.00	1.41	2.75	2.22
SE	Patch	90,688	4	0.75	1.50	0.00	0.00	0.00	0.00
SE	Sand	5,040,496	3	0.00	0.00	0.00	0.00	0.00	0.00
SW	Back	1,796,448	5	0.40	0.55	0.80	1.30	0.00	0.00
SW	Fore	3,257,936	4	4.00	3.74	2.00	3.37	4.00	4.97
SW	Patch	1,068,736	5	1.40	2.07	0.40	0.55	0.20	0.45
SW	Sand	10,429,472	1	0.00	0.00	0.00	0.00	0.00	0.00
Total ± SD (thousands)				**1,273 ± 326**		**348 ± 0.97**		**631 ± 120**	

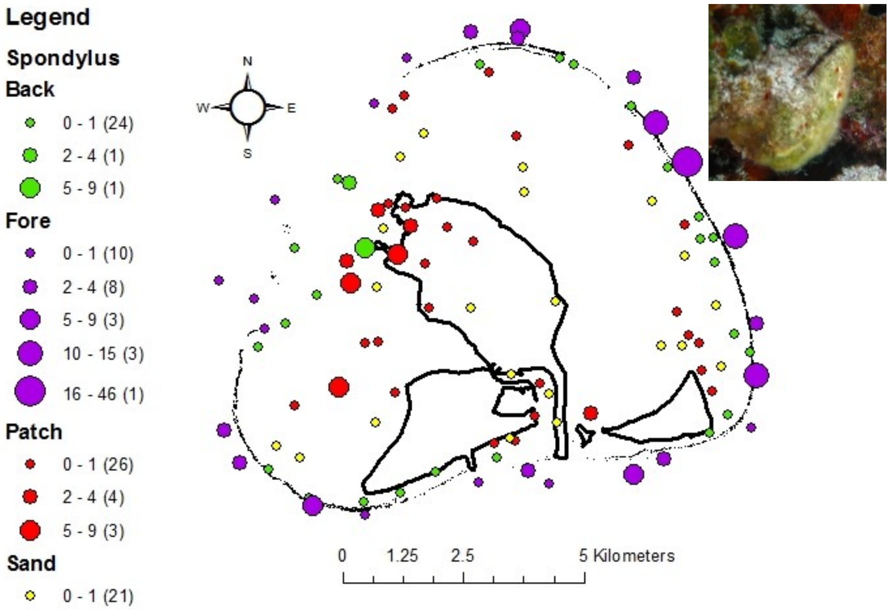

Figure A1: Abundance of *S. violacescens* at survey sites at Midway Atoll, with colors depicting habitat type and size of symbol depicting number of bivalves observed in 100 m^2 transect survey.

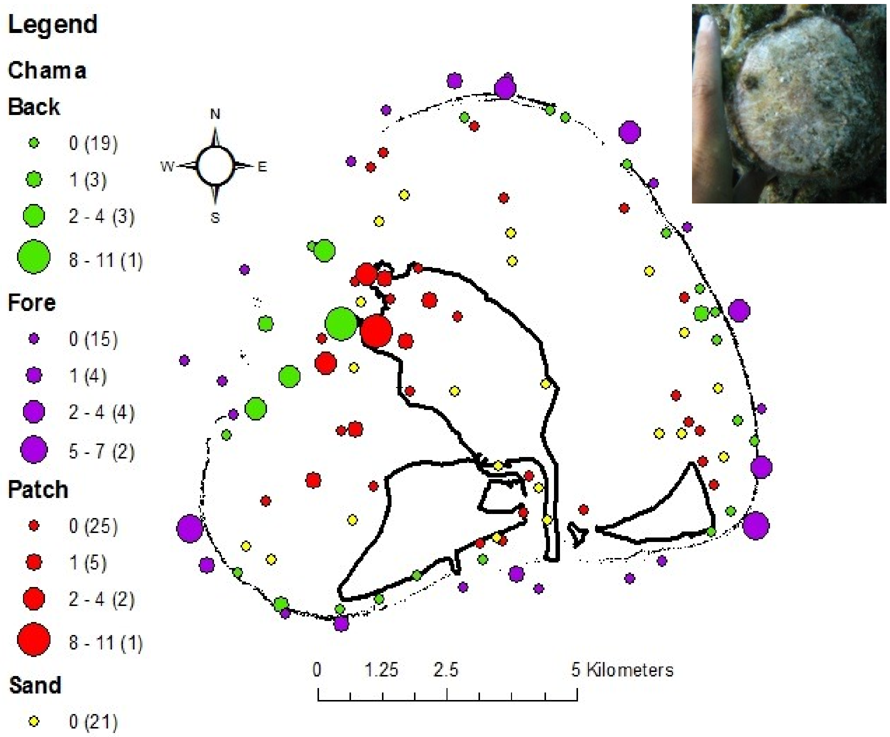

Figure A2: Abundance of *C. limbula* at survey sites at Midway Atoll, with colors depicting habitat type and size of symbol depicting number of bivalves observed in 100 m^2 transect survey.

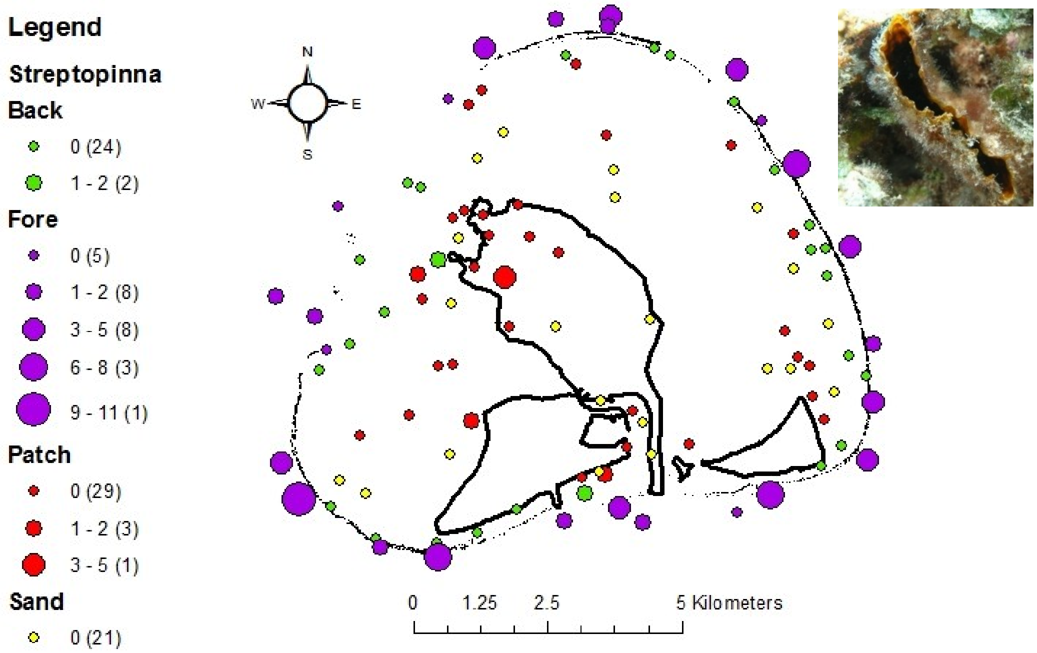

Figure A3: Abundance of *S. saccata* at survey sites at Midway Atoll, with colors depicting habitat type and size of symbol depicting number of bivalves observed in 100 m^2 transect survey.

CHAPTER 2

Introduced, Invisible, or Misidentified: ITS1 Phylogeny Identifies New *Pinctada* Species at Midway Atoll

Introduction:

The Hawaiian Islands are the most isolated land areas in the world, located >700 km from the nearest island (Johnston Atoll) and >3000 km from the nearest continents (Alaska, North America and Japan, Asia) (Coles et al. 1999). The archipelago's isolation in the center of the world's largest and deepest ocean has worked as a barrier to immigrating species, stopping most terrestrial species and severely filtering marine species. Prior to the arrival of Europeans in the late eighteenth century, colonization by new species of non-pelagic marine organisms probably occurred primarily via fouling communities on drifting objects (rafting) and seabirds and settlement of long-lived planktonic larvae transported by ocean currents, as well as fouling communities on the hulls of Polynesian canoes. The few species that reached Hawaii spread throughout the 2,500 km long archipelago and diversified into a marine biota with relatively low species richness but one of the world's highest rates of endemism: about 25% of nearshore fishes (Randall 2007), 25% of red algae (Abbott 1999), 30% of invertebrates other than corals (Kay and Palumbi 1987), and 30% of corals (Maragos et al. 2004).

Development of Hawaii as a crossroads of the Pacific Ocean meant increasing frequency of ship and boat arrivals and increased probability of introductions of non-indigenous marine species.

Introduced species can monopolize energy resources, be voracious predators, outcompete native species, or transmit parasites and diseases that can be passed to humans through the food chain or direct exposure. Because of the serious consequences that can result from nonindigenous introductions, marine species invasions are among the most serious potential perturbations of marine ecosystems (Carlton and Geller 1993). Mechanisms for anthropogenic introductions of marine species include fouling and boring of ships' hulls, ballast water (in planktonic or larval forms) of ships, fisheries/aquaculture introductions (including intentional and hitchhiker species), escape and/or release of ornamental and aquarium species, fouling of marine debris, and reconnection of formerly isolated bodies of water by canal construction (Coles et al. 2002). Most of the approximately 300 introduced marine species known in Hawaii are believed to have been transported via fouling or ballast water of international shipping from the Indo-West Pacific, Eastern Pacific, and North Atlantic (Carlton and Eldredge 2009).

Thousands of marine species worldwide cannot be labeled as native or nonnative due to a lack of systematic, biogeographic, historical, and fossil data and thus are labeled as cryptogenic (i.e., species that are not demonstrably native or introduced, Carlton 1996). Carlton and Eldredge (2009) describe 117 cryptogenic marine species in Hawaii, where origin is often unclear because biologists conducted few surveys before 1900. In fact, thorough studies of Hawaii's reefs, bays, and harbors in the 1990s discovered many new cryptogenic and introduced species (e.g., Coles et al. 1999, 2002, Defelice et al. 1998). Geller et al. (2010) advocated using genetic methods, such as phylogeographic reconstruction based on sequences of DNA "barcoding" markers, to clarify the status of cryptogenic species and to unmask cryptic invasions, in which invaders are mistaken for native or previously introduced species.

Cryptogenic species and cryptic invasions are likely to be particularly common in species that are poorly resolved by external morphology, such as pearl oysters in the genus *Pinctada* (phylum Mollusca, class Bivalvia, order Pteroidea, family Pteriidae). Pearl oysters are of great economic importance, as they are cultured for pearl production, and are widely distributed throughout tropical and subtropical oceans. They are also important filter feeders in coral reef ecosystems and help remove nutrients, plankton, and heavy metal pollutants and maintain water quality. They have also been studied as potential ecosystem indicators and bioremediation agents for heavy metal pollution (Sarver et al. 2003, Gifford et al. 2005, Macfarlane et al. 2005) and eutrophication (Gifford et al. 2004, 2005, 2007).

Taxonomy of pearl oysters has traditionally been based on shell features (shape and color), which are largely influenced by environmental factors and heterogeneity among habitats. Species identification based on morphology is particularly difficult in juveniles because of shell similarity (Wada and Tëmkin 2008, Wang et al. 2004 in Cunha et al. 2011). Several recent studies of *Pinctada* phylogeny using molecular techniques and a variety of genetic markers agree on a general phylogeny (Figure 1), but speculate on two widely distributed species complexes: *P. fucata/martensii/imbricata/radiata* and *P. margaritifera/galtsoffi/mazatlanica* (Yu and Chu 2006: ITS1 and ITS2; Tëmkin 2010: 18S, 28S, and 16S rRNA, and H3 histone; Cunha et al. 2011: COI and 18S rRNA; Masaoka and Kobayashi 2005c: 28S rRNA). None of these phylogenetic studies used specimens from Hawaii.

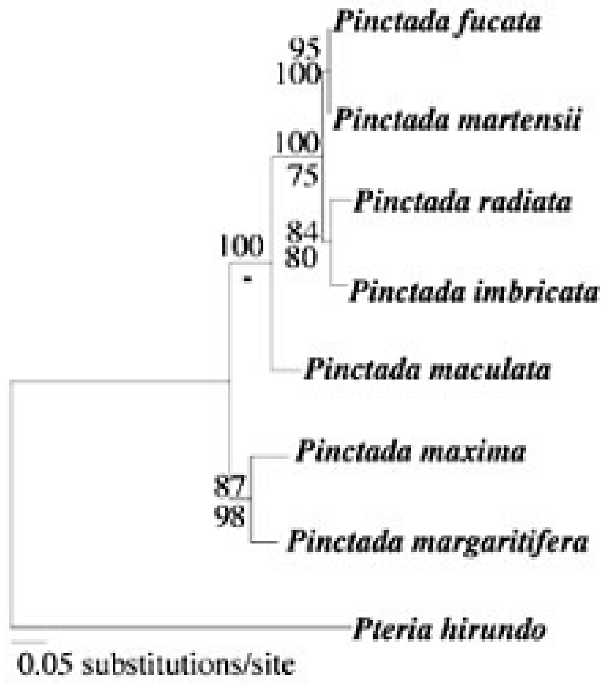

Figure 1: Phylogeny of pearl oyster based on two markers (COI and 18S rRNA), with node labels corresponding to Bayesian posterior probabilities (above branches) and maximum likelihood bootstrap proportions (below branches). Only values above 70% are represented (Cunha et al. 2011).

Two *Pinctada* species are reported from Hawaii (Kay 1979, Severns 2011): *P. radiata* (Leach, 1814) and *P. margaritifera* (Linneaus, 1758) (Figure 2). *P. radiata* grows to about 60 mm and *P. margaritifera* grows to about 290 mm in Hawaii, which is larger than documented in other parts of the Indo-Pacific (Kay 1979, Cernohorsky 1978). Both species are distributed throughout the Hawaiian archipelago, but *P. radiata* historically occurred at high densities in Pearl Harbor, Oahu, and *P. margaritifera* historically occurred at high densities at Pearl and Hermes Atoll in the Northwestern Hawaiian Islands (Walther 1997).

Figure 2: (a) Exterior and (b) interior views of *P. radiata* (left in each photo) and *P. margaritifera* (right in each photo) specimens at the Bishop Museum, in Honolulu, Hawaii (Walther 1997).

Before the arrival of Europeans, native Hawaiians collected *P. radiata* from large beds in Pearl Harbor for food and shell, which they used to make fishhooks and scraper tools and to decorate bowls and god images (Walther 1997). After Captain Cook reached Hawaii in 1778, King Kamehameha directed his divers to collect pearl oysters throughout Pearl Harbor (Wai-Momi) and traded them to Europeans throughout the late 1700s and early 1800s, but, by 1840, deforestation and overgrazing in the mountains of Oahu smothered the oyster beds of Pearl Harbor with silt and debris. The World War II attack on Pearl Harbor in 1941 also caused great ecological damage that probably destroyed any remaining populations of *P. radiata*. Kay (1979) states that *P. radiata* is a common shallow-water species throughout the Hawaiian archipelago, and Stender (2013) reports that *P. radiata* is occasionally found in harbors and bays with freshwater input.

The black-lipped pearl oyster (*P. margaritifera*) is cultured to produce black pearls throughout the Indo-Pacific. Within three years of the discovery of large *P. margaritifera* beds at Pearl and Hermes Atoll (Figure 3) in 1927, divers removed an estimated 100 tons

(~150,000 individuals) from Pearl and Hermes Atoll, primarily for export to the U.S. mainland to make buttons (Galtsoff 1931). After Galtsoff studied the remaining population in 1930 and concluded that it was severely depleted, the Territory of Hawaii prohibited the take, kill, possession, removal, or sale of pearl oysters in Hawaii without a permit. Limited surveys in 1994 and 2000 and large surveys as part of a marine debris removal effort in 2003 found that the abundance of pearl oysters at Pearl and Hermes Atoll (~177 km^{-2}) was similar to the population size in 1930 (~209-349 km^{-2}) and the population therefore likely had not recovered from overexploitation in over 70 years, perhaps due to an Allee effect (Keenan et al. 2006). Rodgers and Sims (2000) estimated that the pearl oyster population at Kaneohe Bay, which they call "one of the few areas in the state where a relatively substantial population remains," numbered about 950 individuals in 1997 (95% confidence intervals of 384-1,538); they found no recruitment and observed evidence of fishing despite legal protection.

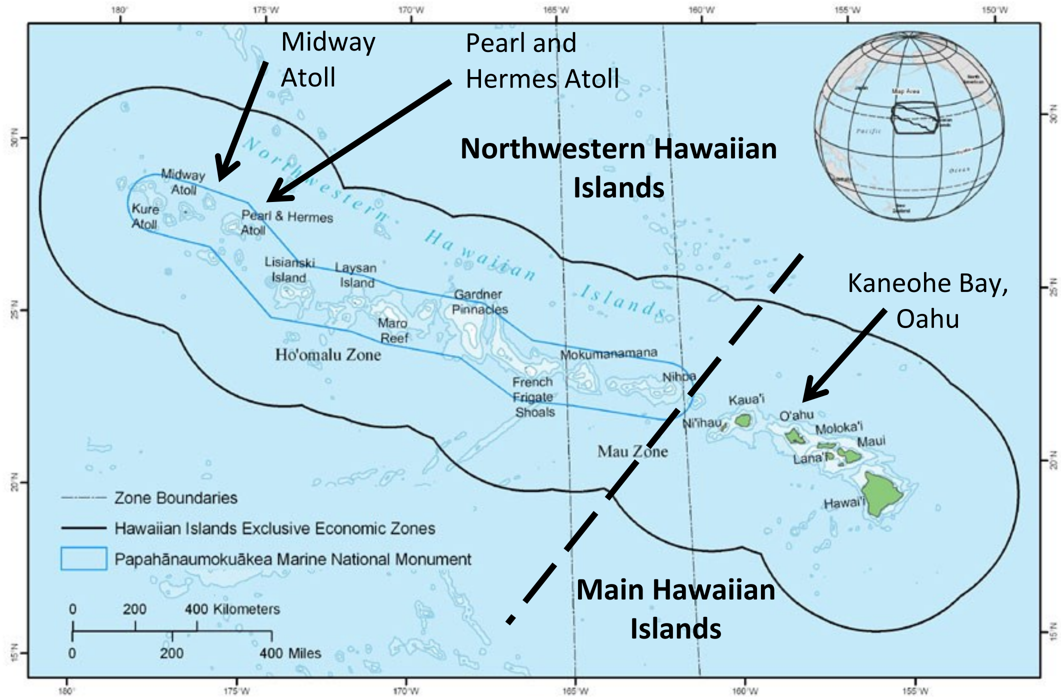

Figure 3: Hawaiian archipelago with Exclusive Economic Zone (black lines), Papahānaumokuākea Marine National Monument borders (blue line), and locations of main and/or studied pearl oyster populations (modified from PMNM 2008).

As part of an effort to describe demography and abundance of *P. margaritifera* at Midway Atoll (Figure 3 and 4) and to explore possible restoration strategies, I measured pearl oyster recruitment throughout Midway's backreef and lagoon and surveyed for adult pearl oysters. I observed over 1400 recruits over four years (2008-2011), but observed only five adult *P. margaritifera* in 108 surveys for bivalves in 2009 and 2011 and another 11 adults during towboarding and other activities in 2008-2011 (McCully, unpublished data). Thirteen of these individuals were observed on patch reefs in the lagoon, while two were on the seawall inside the main harbor and one was found on the forereef. Keenan (2007) also concluded that *P. margaritifera* is extremely rare at Midway Atoll as she found only two individuals during annual towboarding surveys in 2003-2006. The only other observations of *Pinctada* at Midway Atoll include Defelice et al. (1998), who observed *P. radiata* at piers inside the harbor and on the northeast side of Sand Island, and Rapid Ecological Assessment surveys, which found 3 *P. margaritifera* in 57 surveys at five forereef and one lagoon site in 2002-2008 (NOAA CRED, unpublished data).

Because morphological identification of *Pinctada* recruits is often difficult and so many more recruits than adults were observed at Midway, I use molecular techniques and phylogenetic reconstruction to identify *Pinctada* recruits collected at Midway Atoll.

Materials and Methods:

Sample Collection

I collected 43 specimens in 2010 and 55 specimens in 2011 from a bivalve recruitment experiment at several backreef and lagoon sites, including some that were used in a growth experiment. 2010 specimens were preserved in denatured alcohol (ethanol with methanol and other additives to reduce palatability); 2011 specimens were preserved in 95% or 100% ethanol. 17 specimens (6 from 2010, 11 from 2011) were successfully sequenced for nuclear marker ITS1 (Table 3). These specimens range from 2.1 to 31.9 mm in dorsoventral measurement (DVM) and include a variety of color patterns. The collection sites are depicted in Figure 4.

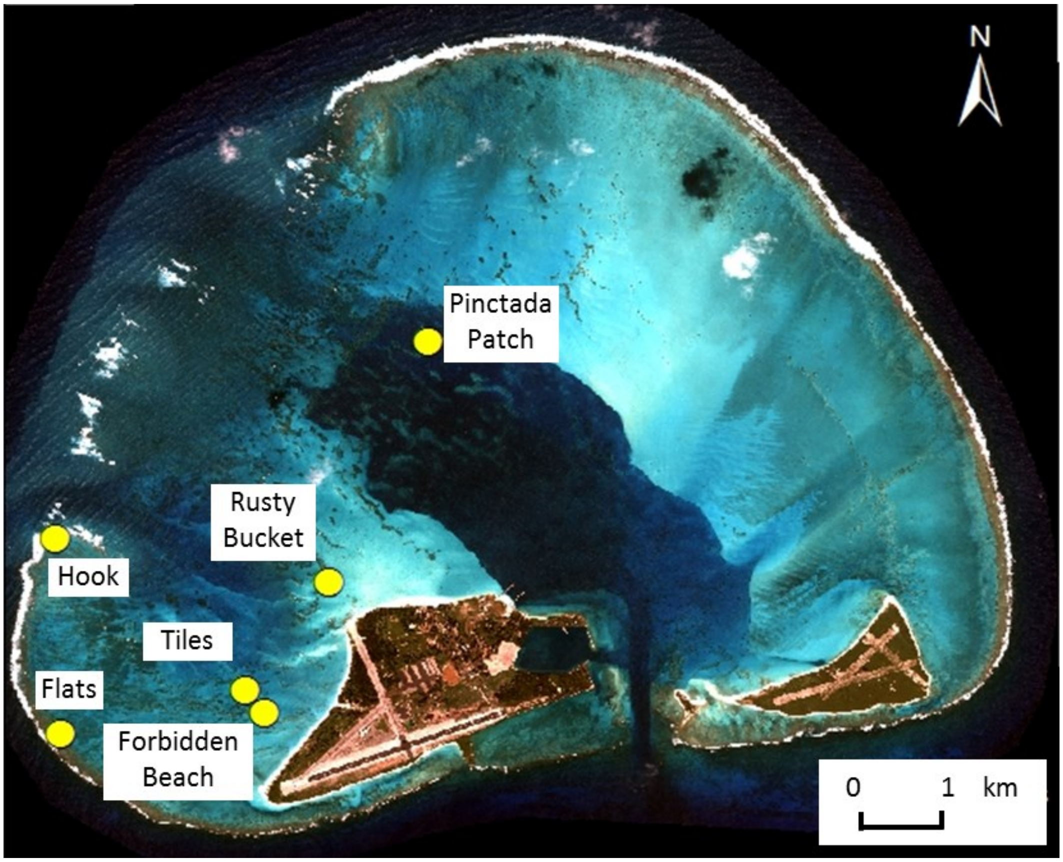

Figure 4: IKONOS satellite image of Midway Atoll (NOAA 2003), with collection sites labeled.

Genetic Analysis

Each specimen's shell was photographed, measured, and described before it was opened and a small piece of muscle tissue (approximately 8 mm^3) was removed. DNA was extracted according to the spin-column protocol for purification of DNA from animal tissue in Qiagen DNeasy Kit (Qiagen). Quality of purified DNA was visualized on 0.8% agarose gel. Quantity of purified DNA was determined using a Nanodrop 1000 spectrophotometer machine (Nanodrop Technologies).

The nuclear marker ITS1 with partial 18S and 5.8S rRNA gene segments was amplified using polymerase chain reaction (PCR) using primers sp-1-5 (5' CACACCGCCCGTCGCTACTA 3') and sp-1-3 (5' ATTTAGCTGCGGTCTTCATC 3') in Chu et al.

(2001). PCR reactions were performed in 25 µl solution containing 17 µl of ddH2O, 2.5 µl of DNA polymerase 10x buffer (New England Biolabs), 0.75 µl of 50 mM MgCl2, 0.5 µl of 10 mM dNTP (Qiagen), 1 µl of each of the 10 µM primers (Operon), 2 µl of DNA template, and 0.25 µl of 5u/µl Taq DNA polymerase (New England Biolabs). The cycling profile was: 90 seconds at 94°C; 33 cycles of 20 seconds at 94°C, 30 seconds at 46.8°C, and 30 seconds at 72°C; and finally 5 minutes at 72°C.

The size and quality of PCR products were visualized on 0.8% agarose gel. Prior to sequencing, PCR products were purified using ExoSAP-IT (Affymetrix). Double-stranded PCR products were sequenced in both directions using the same primer pairs as in the PCR reaction. The cycle sequencing reactions were performed using BigDye Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's instructions (Invitrogen). The resulting products are analyzed using a 3730xl DNA Analyzer (Invitrogen).

Phylogenetic Reconstruction

39 ITS1 sequences from 11 *Pinctada* species and one ITS1 sequence from *Pteria penguin* (as outgroup) were obtained from GenBank (Table 1) and included in this analysis. The multiple sequence alignment was made using ClustalX2 (Larkin et al. 2007). All sequences were trimmed to the boundaries of the shortest aligned sequences. The largest gaps in aligned sequences were removed since each gap has an undue influence on the tree for a single evolutionary event (Baldauf 2003).

Table 1: GenBank sequences for *Pinctada* species that were used in this study.

Code	**Species**	**#**	**Length (bp)**	**Collection Location**
AY877498	*P. albino*	1	509	Port Stephens, Australia
AY877499	*P. albina*	2	505	Port Stephens, Australia
AY877496	*P. chemnitzi*	1	534	Daya Bay, China
AY877497	*P. chemnitzi*	2	533	Hong Kong, China
AB214204	*P. fucata*	1	401	Myanmar (hatchery)
AB214208	*P. fucata*	2	402	Perth, Australia
AB214209	*P. fucata*	3	401	Cambodia
AB214212	*P. fucata*	4	401	China: Hainan (hatchery)
AB214219	*P. fucata*	5	402	Kagoshima, Amami, Japan
AY877512	*P. fucata*	6	495	Sanya Bay, China
AY877577	*P. fucata martensi*	1	497	Mie Prefecture, Japan
AY877578	*P. fucata martensi*	2	497	Mie Prefecture, Japan
AB214197	*P. imbricata*	1	402	Florida, USA
AB214198	*P. imbricata*	2	399	Florida, USA
AB214199	*P. imbricata*	3	404	Florida, USA
AY877569	*P. imbricata*	4	495	Port Stephens, Australia
AY877571	*P. imbricata*	5	497	Port Stephens, Australia
AB214194	*P. maculata*	1	403	Amami, Kagoshima, Japan
AB214195	*P. maculata*	2	404	Amami, Kagoshima, Japan
AB214196	*P. maculata*	3	406	Amami, Kagoshima, Japan
AB214188	*P. margaritifera*	1	447	Okinawa, Japan
AB214189	*P. margaritifera*	2	447	Okinawa, Japan
AY491420	*P. margaritifera*	4	610	Beihai, China
AY877500	*P. margaritifera*	5	542	Sanya, Hainan Island, China
AY877501	*P. margaritifera*	6	542	Sanya Bay, Hainan Province, China
AY877502	*P. margaritifera*	7	543	Sanya, Hainan Island, China
AY883845	*P. margaritifera*	8	542	Sanya Bay, Hainan Province, China
AY172344	*P. martensi*	1	705	Sanya, Hainan Island, China
AB214223	*P. martensi*	2	400	Nagasaki, Tsushima, Japan
AB214237	*P. martensi*	3	403	Ishikawa, Anamizu, Japan
AY172345	*P. maxima*	1	777	Sanya, Hainan Island, China
AB214185	*P. maxima*	2	480	Philippines
AB214186	*P. maxima*	3	482	Philippines
AB214187	*P. maxima*	4	480	Philippines
AY192147	*P. nigra*		704	Sanya, Hainan Island, China

AB214200	*P. radiata*	1	400	Unknown
AB214201	*P. radiata*	2	401	Unknown
AB214202	*P. radiata*	3	401	Unknown
AB214203	*P. radiata*	4	400	Unknown
AY877503	*Pteria penguin*		478	Sanya, Hainan Island, China

I used two phylogenetic approaches: maximum likelihood (ML) and Bayesian inference (BI). I first determined the appropriate evolutionary model using jModelTest (Darriba et al. 2010, Guindon and Gascuel 2003). Although jModelTest indicated that the appropriate model was TVM+G (transversional model with a gamma-shaped distribution of rates across sites), this model was not available on the software used for phylogeny reconstruction. I instead used the second best model (ΔAIC = 0.60) GTR+G (a General Time Reversible model with a gamma-shaped distribution of rates across sites).

Based on JModelTest results, the ML tree was reconstructed using RAxML (Stamatakis et al. 2008) and a bootstrapping test (Felsenstein 1985) was conducted using 100 pseudoreplicates.

Bayesian analysis was implemented using MrBayes v. 3.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). The GTR+G model was assumed while other settings were kept default. Markov chains were run for one million generations with 0.25% pre-burn-in states, sampled every 100 generations resulting in 10,000 trees. Posterior probabilities were used to assess the robustness of the consensus tree.

Phylogenies were visualized using FigTree (Rambaut 2012).

Morphological Analysis

I examined each shell of the sequenced samples for morphological characteristics used in *Pinctada* taxonomy described in Hynd (1955), Masoaka and Kobayashi (2005b), and Colgan and Ponder (2002), including:

- White porcellanous patches on non-nacreous patches of *P. maculata* shells that are not present for *P. fucata* (Hynd 1955)
- Golden tint of nacre of *P. maculata*, but not *P. fucata* (Hynd 1955)
- Sparse, regularly distributed growth processes in *P. maculata* in contrast to dense growth processes in *P. fucata* (Masaoka and Kobayashi 2005b)
- Smaller size of *P. maculata* than *P. imbricata* (Colgan and Ponder 2002)

Results:

Phylogenetic Reconstruction

After gaps were deleted, the aligned length of ITS1 in this study consists of 273 sites, of which 210 were variable and 128 were parsimony informative. This is shorter than the 529-bp ITS1 sequence used in Yu and Chu (2006) because I included several GenBank sequences for specimens from other locations (≥399 bp) and two of the Midway sequences were 319 and 366 bp before alignment.

In a distance matrix consisting of the percentages of identical bases in all sequences of all possible pairs of taxa (Table 2), the 17 Midway samples had the highest percentage of identical nucleotides with themselves (95.1-100%, average 99.1% ± 1.1% standard deviation) and with the three *P. maculata* sequences (95.5-99.3%, 98.1 ± 0.8%). The divergence between the Midway samples and *P. maculata* is much less than and does not overlap with the divergence between taxa. Thus, the Midway samples and *P. maculata* have diverged much less than any other pair of different taxa. For instance, the percentage of identical nucleotides between *P. maculata* and the *P. fucata/martensii/imbricata/radiata* species complex is 87.8-90.0% (89.3% ± 0.5%). The percentage of identical nucleotides between the Midway samples and the two previously reported Hawaiian *Pinctada* species is similar or less than the percentage of identical nucleotides between different species: *P. radiata* (87.7-90.0%, 89.7% ± 0.6%) and *P. margaritifera* (63.0-64.9%, 64.5% ± 0.4%). Thus, the Midway samples are much more closely related to *P. maculata* than to any of the other taxa, including *P. radiata* and *P. margaritifera*.

Table 2: Distance matrix for ITS1 sequences. Each number in the table is the average percentage of sites with the same base in all sequences used of the two given taxa. Within-taxon distances are not given for taxa with only one sequence. Labels are: 1. Midway samples (n =17); 2. *P. maculata* (n = 3); 3. *P. fucata martensii* (n = 2); 4. *P. fucata* (n = 6); 5. *P. imbricata* (n = 5); 6. *P. martensii* (n = 3); 7. *P. radiata* (n = 4); 8. *P. albina* (n = 2); 9. *P. chemnitzi* (n = 2); 10. *P. nigra* (n = 1); 11. *P. margaritifera* (n = 7); 12. *P. maxima* (n = 4); 13. *Pteria penguin* (n = 1).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1.	99.1												
2.	98.0	98.1											
3.	89.6	89.7	98.5										
4.	89.3	89.1	98.3	98.0									
5.	89.5	89.2	98.2	97.5	99.0								
6.	90.0	89.6	99.1	98.1	99.0	99.7							
7.	89.6	89.3	98.9	98.2	98.5	99.3	99.0						
8.	80.6	80.6	79.5	78.4	79.5	80.2	80.2	98.5					
9.	80.1	80.1	80.3	78.5	79.6	80.3	80.3	90.2	99.6				
10.	77.2	77.2	77.1	75.6	77.1	77.5	77.5	93.6	88.8	-			
11.	64.4	64.4	63.9	63.5	63.1	63.9	63.5	59.4	58.9	57.1	97.9		
12.	64.6	65.3	63.0	62.6	62.2	63.0	63.0	58.9	58.7	56.9	91.6	96.2	
13.	39.7	40.2	40.6	40.2	40.6	40.6	40.2	38.0	39.4	37.5	34.9	35.2	-

Phylogenetic trees were reconstructed by two approaches: Maximum Likelihood (Figure 5) and Bayesian inference (Figure 6). The likelihood value (-Ln[L]) is -1736.8 for the ML tree.

The two approaches resulted in slightly different trees (Figures 5 and 6), but the Midway samples were always clustered with the three *P. maculata* sequences (ML bootstrap value of 72% and BI posterior probability of 68%). Thus, the Midway samples clustered with *P. maculata* in 72% of the ML replicate trees, and the BI tree shows that there is a 68% probability that the Midway samples and *P. maculata* form a monophyletic group, given the model and data.

In both trees, *P. margaritifera* and *P. maxima* form a single clade and *P. fucata martensii*, *P. fucata*, *P. imbricata*, and *P. radiata* also form a separate clade (henceforth referred to as the *P. radiata* clade). These features are also consistent with the previously published phylogenies (Figure 1, Yu and Chu 2006, Tëmkin 2011, Cunha et al. 2011, Masaoka and Kobayashi 2005d in Wada and Tëmkin 2008).

In both trees, the Midway samples, *P. maculata*, *P. maxima*, and *P. margaritifera* form a single clade, which is different from the previously published phylogenies.

The trees differ in the placement of *P. nigra*, *P. chemnitzii*, and *P. albina*, which are not always included in the previously published phylogenies.

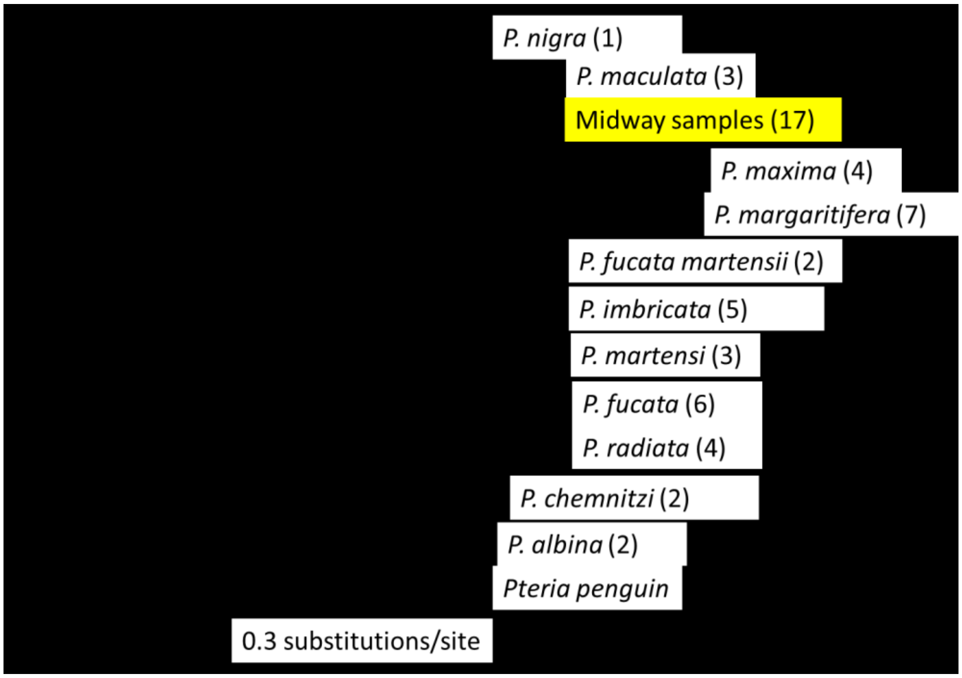

Figure 5: Maximum likelihood tree based on ITS1 data set. Numbers near nodes represent bootstrap values. Numbers after taxonomic names are numbers of specimens/sequences included for each taxon. Branch lengths are proportional to percent sequence difference.

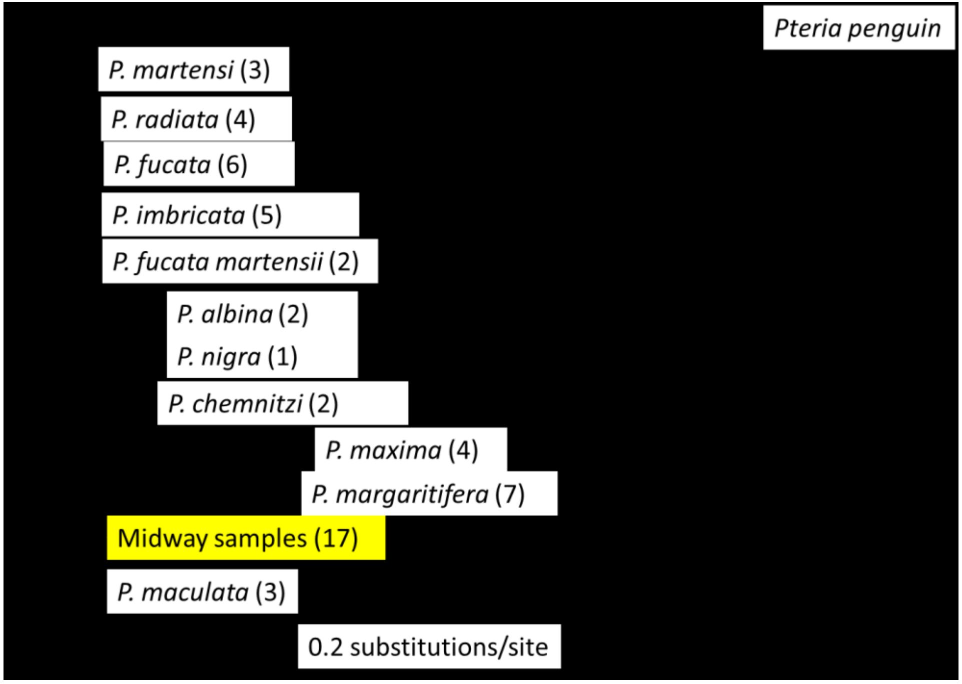

Figure 6: Bayesian inference tree based on ITS1 data set. Numbers near nodes represent posterior probability. Numbers after taxonomic names are numbers of specimens/sequences included for each taxon. Branch lengths are proportional to percent sequence difference.

Morphological Analysis

Hynd (1955) distinguished *P. maculata* from *P. fucata* by *P. maculata*'s white, porcellanous patches on non-nacreous margin and golden-tinted nacre on the interior of the shell. 11 of the 17 Midway pearl oyster recruits genetically identified as *P. maculata* had white opaque patches, including four specimens in which large white patches resembled a white band (Table 3 and Figure 7). Nine of the ten specimens larger than 12 mm DVM had white patches or bands, while two of the seven specimens <12 mm had white patches or bands, which suggests that these white patches usually develop only in larger, older individuals. I did not observe a golden tint in the nacre of any of the recruits.

Table 3: Description of *Pinctada* specimens collected at Midway Atoll and successfully sequenced for ITS1. Collection sites for specimens that were used in a growth experiment include both the original recruitment location and the indication "(Growth)". Original recruitment site is not available for two specimens. Asterisk indicates specimen had white porcellanous patches inside shell characteristic of *P. maculata* and double-asterisk indicates specimen has white porcellanous band parallel to margin.

Specimen	**Date Collected**	**Site Collected**	**DVM (mm)**	**Description**
MDY-01	8/25/2010	Pinctada Patch	18.4	white with black inner stripes & broken growth processes*
MDY-02	8/14/2010	Flats	11.2	dark red with white rays & umbo
MDY-03	?/2010	?	9.5	gray with white umbo & transparent edge*
MDY-04	8/14/2010	Flats	7.6	gray/brown with black ring, white umbo
MDY-05	8/14/2010	Hook	15.0	gold rays, few dark rings on front*
MDY-06	8/14/2010	Flats	14.6	red rings, long red growth processes and white umbo*
MDY-07	8/9/2011	Pinctada Patch	18.5	dark brown to almost black with growth processes and copper right at umbo
MDY-08	8/9/2011	Forbidden Beach	20.4	black with white rays and growth processes*

MDY-09	8/9/2011	Forbidden Beach	11.2	white with pink & brown speckles and tiny growth processes
MDY-10	8/9/2011	Forbidden Beach	7.4	all dark mahogany brown with small growth processes
MDY-11	9/3/2011	Rusty Bucket	7.5	white umbo with brown stripes and growth processes
MDY-12	9/17/2011	Forbidden Beach (Growth)	17.6	white*
MDY-13	9/17/2011	Pinctada Patch (Growth)	13.3	dark with white umbo & overgrown by algae along edge*
MDY-14	7/18/2011	Tiles	14.0	white with dark patches at edge & a little filamentous orange algae**
MDY-15	9/5/2011	Rusty Bucket (Growth)	19.4	red rays with white umbo**
MDY-16	8/22/2011	Pinctada Patch (Growth)	8.3	white**
MDY-17	9/17/2011	Pinctada Patch (Growth)	16.8	white with dark patches, translucent in places**

Masaoka and Kobayashi (2005b) cite an older identification key in Japanese for *Pinctada* (Takemura and Okutani 1958) that described different patterns of processes (scales) on the external shells of *P. maculata* and *P. fucata*: they are densely packed in *P. fucata*, but sparsely distributed at regular intervals in *P. maculata*. Unfortunately, the difference is unclear in the photos Masaoka and Kobayashi (2005b) provide, so it's difficult to use this criterion to identify the Midway specimens.

Masaoka and Kobayashi (2005b), Colgan and Ponder (2002), and Hynd (1955) point out that *P. maculata* is much smaller and grows more slowly than *P. fucata* and *P. imbricata* (both in the *P. fucata/imbricata/radiata/martensii* species complex). However, the largest sequenced Midway recruit is 20.4 mm, which is considerably smaller than the maximum size of 55 mm for *P. maculata* and 90 mm for *P. fucata* (Hynd 1955), so it is likely that these

Midway individuals are not yet full-grown and may not yet display many of the characteristics often used for identifying pearl oysters.

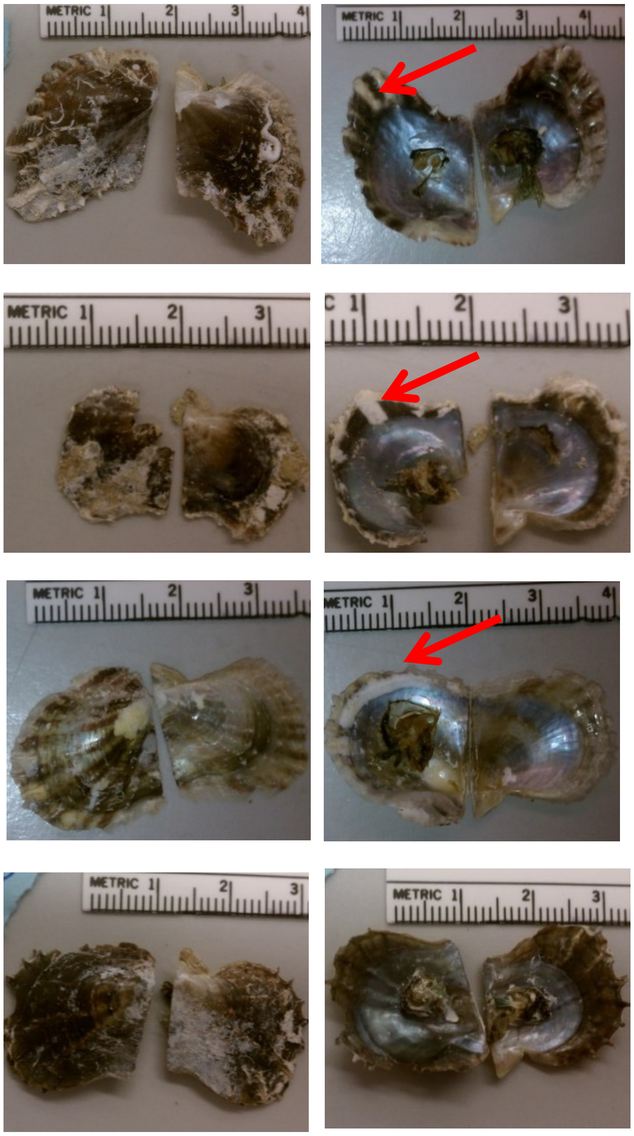

Figure 7: Exterior (right) and interior (left) views of Midway *Pinctada* shells: (a) MDY-08 with white porcellanous patches, (b) MDY-13 with white porcellanous patches, (c) MDY-15 with white porcellanous band, and (d) MDY-07 with no white porcellanous patches or band. Arrows indicate patches and band. Ruler in each photo indicates centimeters and millimeters.

Discussion:

A phylogenetic reconstruction of the genus *Pinctada* using ITS1 sequences from 17 Midway specimens and 39 positively-identified individuals from eleven *Pinctada* species and one outgroup species (GenBank) shows that *Pinctada* recruits at Midway are most closely related to *P. maculata*, a species not previously reported in the Hawaiian archipelago. Both maximum likelihood and Bayesian inference phylogenies show that the Midway samples and *P. maculata* sequences form a monophyletic group separate from the two previously reported Hawaiian species (*P. margaritifera* and *P. radiata*). Maximum likelihood bootstrap values of 72% and the Bayesian posterior probability of 0.68 show that the Midway samples and *P. maculata* form a reliable and consistent clade. Most of the shells also include a morphological characteristic of *P. maculata* used to differentiate it from the *P. radiata* species complex.

Much less is known about *P. maculata* than about the *Pinctada* species used in pearl culture (e.g., *P. margaritifera, P. maxima, P. fucata/martensi*). *P. maculata* is recorded from many locations throughout the tropical Pacific (e.g., Japan, Philippines, French Polynesia, Samoa, Australia, New Zealand, South Africa, Papua New Guinea, Guam, and many smaller island nations) (Figure 8, GBIF 2013). Hynd (1955) reports that largest *P. maculata* in the Australian Museum collection measured 55 mm DVM. Niquil et al. (2001) described *P. maculata* as a pest species settling on pearl culture long lines at Takapoto Atoll in about five times the density as the cultured *P. margaritifera*. Strack (2008: p.31) states that *P. maculata* produces tiny golden pearls, rarely larger than 5 mm. These pearls, called “poe pipi” in Maori language and commonly “pipi” pearls, are mainly found in the Cook Islands.

Pearl culture trials in the Cook Islands used *P. maculata* in the 1950s, but pearl culture since then has focused exclusively on black pearls from *P. margaritifera*.

Figure 8: Reported occurrences of *P. maculata* from Global Biodiversity Information Facility (GBIF 2013).

The only previous record of small pearl oysters at Midway Atoll is in a brief survey focused on introduced marine invertebrates conducted in 1997 by DeFelice et al. (1998). They report that no previous systematic marine invertebrate survey of Midway had been conducted prior to their study and nearly quadrupled the number of marine invertebrates previously reported from Midway. They identified bivalves on pier pilings inside the harbor and on the northeast side of Sand Island as *P. radiata*, but gave no descriptions of them.

Three hypotheses can explain the absence of *P. maculata* records from the Hawaiian archipelago:

- The small pearl oyster present in Hawaii, identified morphologically as *P. radiata*, is actually *P. maculata*.

- *P. maculata* is a native species in Hawaii, in addition to *P. radiata* and *P. margaritifera*, but has not yet been reported.
- *P. maculata* has been introduced to Midway and perhaps other parts of Hawaii, but has either been incorrectly identified as *P. radiata* or not yet observed. This scenario is a cryptic invasion, as described by Geller et al. (2010).

All three scenarios seem feasible because invertebrate faunas of Midway Atoll and the other Northwestern Hawaiian Islands are poorly known, both *P. maculata* and *P. radiata* are small and cryptic, and *P. maculata* is morphologically very similar to and a sister species of the *P. radiata* species complex. During 108 bivalve surveys throughout Midway's shallow benthic habitats (McCully, unpublished), I observed only 2 small *Pinctada* specimens similar to those used in this study. Both were on coral rubble lying on top of primarily soft substrates inside the lagoon. Excluding those settling on bivalve recruitment collectors and on coral settlement tiles, most naturally occurring *Pinctada* recruits I observed at Midway were on pieces of rubble (including bivalve shells) in sediment samples collected for other purposes (Potts, unpublished). The *Pinctada* specimens were usually not seen until the rubble was examined later. I have observed small pearl oysters (*Pinctada* spp.) ranging from 0.1-22 mm and morphologically consistent with being *P. radiata* or *P. maculata* and large *Pinctada* (16.7 cm - 23.8 cm) which have been positively identified as *P. margaritifera* adults.

It is quite possible that *P. maculata* has been mistaken for *P. radiata* either throughout the history of Hawaiian malacology or since a more recent introduction because the two species are morphologically very similar. Kay's (1979) description of Hawaiian *P. radiata* and Cernohorsky's (1978) description of *P. maculata* in his *Tropical Pacific Marine*

Shells overlap greatly: small size ≤ 60 or 65 mm; oval shape; larger left valve; scaly ridges on exterior; and intertidal habitat. Although their descriptions of interior and exterior shell color vary slightly, color is notoriously variable among *Pinctada* individuals and among observers. Similarly, in Hynd's (1955) taxonomic revision of Australian *Pinctada*, six of nine qualitative characteristics state that *P. maculata* is similar to *P. fucata* (part of *P. radiata* species complex). Hynd (1955) differentiated *P. maculata* from *P. fucata* in both the text of his species descriptions and in his key to Australian pearl oyster species by: *P. maculata* having white porcellanous patches on the non-nacreous margin (which he says are frequently absent in small specimens), golden tint of nacre, and opaque, white or horny growth processes. These characteristics are based on Australian specimens and are not part of the description of *P. maculata* in Cernohorsky (1978). Similarly, Ranson (1961) says that interior and exterior colors of *P. maculata* shells are very variable and that the nacre can be yellow, yellow-brown, or even white, but does not mention the white porcellanous patches.

Kay (1979) noted that Hynd (1955) suggested that Hawaiian *P. radiata* may be a synonym of *P. maculata*. Small Hawaiian pearl oysters was originally named *Avicula pallida* and *A. nebulosa* by Conrad (1837) and later described by Reeve (1857). Jameson (1901) and Hynd (1955) considered the two species synonymous. Hynd (1955) stated that there is a "strong possibility" that both species are synonymous with *P. maculata* due to Reeve's (1857) description of the golden hue of the internal nacre, a characteristic of *P. maculata*. This cannot be confirmed since the type specimens of both *A. pallida* and *A. nebulosa* have been lost (Hynd 1955). In response to Hynd (1955), Ranson (1961) asserted that the Hawaiian species *A. pallida* and *A. nebulosa* are actually synonyms for *P. radiata,* relying on such characteristics as shell size, muscle, and prodissoconch (larval shell) in Hawaiian specimens and in descriptions of the type specimens. This work (Ranson 1961) appears to be the primary reason the small Hawaiian pearl oyster is called *P. radiata*.

P. maculata and the *P. radiata* species complex are closely related in all *Pinctada* phylogenies that include *P. maculata* (Tëmkin 2010, Cunha et al. 2011, Masaoka and Kobayashi 2005c). In Japan, the two species are able to hybridize (Masaoka and Kobayashi 2005b) and are karyotypically similar with the same total number and very similar counts of types of chromosomes (Wada and Komoru 1985).

The second hypothesis that *P. maculata* is a distinct species native to Midway and other reefs of the Hawaiian Archipelago, but not yet recognized, is also plausible due to the limited surveys of smaller invertebrates on NWHI reefs. Apart from one survey at Midway Atoll in 1997 (DeFelice et al. 1998), a series of Rapid Ecological Assessments (2002, 2003, 2004, and 2008) have examined a few belt transects on each reef. For example, these surveys together recorded only six bivalve species, including *P. margaritifera*, on Midway Atoll, and six 2008 transects on Midway found only 11 bivalves of four species (NOAA CRED, unpublished data).

Although it is less likely that *P. maculata* would not be detected in the Main Hawaiian Islands due to the extended biological work there over more than a century, *P. maculata* may be one of several Indo-Pacific species restricted primarily to the NWHI, such as the Japanese angelfish (*Centropyge interrupta*), blotcheye soldierfish (*Myripristis murdjan*), and *Acropora* corals (Grigg et al. 2008). Hourigan and Reese (1987) suggested that these fishes reached the NWHI from the West Pacific via the Kuroshio extension of the North Pacific Equatorial Current, while Grigg (1981) suggested that the *Acropora* corals originate from Johnston Atoll (Grigg 1981), 720 km southwest of the French Frigate Shoals, via the Subtropical Countercurrent and Hawaiian Lee Countercurrent (Friedlander et al. 2008); this is supported by an ocean circulation model simulating larval transport from Johnston Atoll to French Frigate Shoals (Kobayashi 2006). *P. maculata* is not known from Johnston Atoll, so *P. maculata* probably did not come from Johnston Atoll. However, *P. maculata* is found throughout the Indo-West Pacific (Figure 8, GBIF 2013), so it may have reached Midway via the Kuroshio extension of the North Pacific Equatorial Current.

The third hypothesis of a recent introduction of *P. maculata* to Midway Atoll also seems extremely feasible because Midway was a major base for naval operations from the late 1930s through the 1980s. Many ships coming to Midway from all over the Pacific may have carried planktonic stages in their ballast water and fouling communities on their hulls. Analysis of first reports of introduced and cryptogenic species at Pearl Harbor suggested that introduction rates peaked during the 1940s with the greatly increased naval activity as part of World War II (Coles et al. 1999). Naval ships visiting Midway frequently came from locations with *P. maculata*, such as Australia, New Zealand, Guam, the Philippines, and Japan (Figure 8). However, surveys of introduced species at Midway have found only six such species: two bryozoans, one barnacle, one hydroid, one polychaete, and one fish (DeFelice et al. 1998; Godwin et al. 2006; Godwin, pers. comm.).

P. maculata could have been introduced to Midway either as larvae in ballast water or as part of the fouling community on ships or on marine debris. The fouling community on an army transport vessel from Hawaii and/or the Marshall Islands sampled in the Puget Sound (Washington) in 1952 included *P. nebulosa*, *P. pallida*, and *P. galtsoffi* (Ranson 1961 lists *Avicula nebulosa* and *A. pallida* as synonyms for *P. radiata* and *P. galtsoffi* as a synonym for *P. margaritifera*) (Eyerdam 1959, in Carlton 1987).

The small pearl oysters (*Pinctada* spp.) collected at Midway Atoll in the Northwestern Hawaiian Islands are genetically almost identical to *P. maculata*, a species not previously reported from the Hawaiian Archipelago. As a small and cryptic species, *P. maculata* may have been incorrectly identified in Hawaii as *P. radiata*, may be native to Hawaii but not yet recognized, or may be an unreported introduced species. This species is established, while relatively rare and cryptic, at Midway Atoll. The extended settlement of new recruits to bivalve recruitment collectors over several years (McCully, unpublished data) suggests the population is self-maintaining. This study highlights the importance of molecular techniques for identifying species and studying connectivity in conservation work.

Further studies to confirm this identification should use other genetic markers with sequences available in GenBank for *Pinctada* species, such as COI, 18S rRNA, 29S rRNA, or 16S rRNA. It will be important to determine whether *P. maculata* exists on other Hawaiian reefs by using molecular techniques to identify small pearl oysters, including those identified as *P. radiata* using morphological characters alone. Although its low density and small size suggest its impacts on reef ecosystems are small, further investigations should determine whether Hawaiian individuals are reproducing and the population is self-sustaining. If *P. maculata* is shown to be an introduced species, the Papahanaumokuakea Marine National Monument, which manages the Northwestern Hawaiian Islands, may want to consider taking measures to prevent the dispersal of *P. maculata* from Midway to other Hawaiian reefs via ballast water and fouling, although it may still be able to move along the Hawaiian archipelago through larval dispersal. The potential expansion of its range and abundance should be considered in the context of environmental protection and long-term management of marine habitats throughout the Hawaiian archipelago.

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CHAPTER 3

Integrating Mathematics into the Undergraduate Biology Curriculum:

A Review of Biology Education Research Literature and an Experiment

Introduction:

"Given the profound changes in the nature of biology and how biological research is performed and communicated, each institution of higher education … should consider the importance of building a strong foundation in mathematics and the physical and information sciences to prepare students for research that is increasing interdisciplinary in character."

- BIO2010: Transforming Undergraduate Education for Future Research Biologists (2003), National Research Council

For more than 25 years, the scientific community, senior science educators, and public policy leaders have called upon colleges and universities to better prepare their undergraduates in science and technology for the difficult social, economic, and environmental challenges of the 21st century in over 19 national reports (summarized in AAAS 2011). Many of these reports have focused on bridging disciplines because many of the most exciting discoveries in the last 50 years occurred at intersections of established disciplines, such as in bioinformatics and biochemistry. In particular, many of these reports call for improved teaching of quantitative skills, including mathematical, statistical, and computational skills, in undergraduate biology curricula. For example, the AAAS (2011) *Vision and Change* report concludes "studying biological dynamics requires a greater emphasis on modeling, computation, and data analysis tools than ever before" and recommends "all students should understand how mathematical and computational tools describe living systems."

Over the last decade, biology and mathematics researchers, instructors, and education researchers have focused on helping students understand the growing relevance of quantitative science in addressing life science questions due to the emphasis on quantitative tools in the 2003 report *BIO2010: Transforming Undergraduate Education for Future Research Biologists* and the 2011 report *Vision and Change in Undergraduate Biology Education: A Call to Action*. These studies focus on research-oriented biology majors, all biology majors, and all undergraduates taking biology courses because “understanding the nature and application of mathematics (including statistics) is essential to general scientific literacy as well as biological research and should be included in the undergraduate biology curriculum” (Chevalier et al. 2010). Although these reports were funded by American institutions, the concern for improving quantitative skills in life science education is not limited to the United States (Matthews et al. 2010).

Biologists need knowledge and experience of mathematics and other disciplines because biological research is becoming more quantitative and requires more interdisciplinary collaborations (Bialek and Botstein 2004) and because biologists must have at least a basic understanding of other disciplines to contribute to good policy (Brent 2004). In *A New Biology for the 21st Century*, NRC (2009) argues “Biological research is in the midst of a revolutionary change due to the integration of powerful technologies along with new concepts and methods derived from inclusion of physical sciences, mathematics, computational sciences, and engineering … [and] will require researchers with both depth of knowledge in a specific discipline and highly developed computational and quantitative skills.” Robeva and Laubenbacher (2009) concluded that “the new generation of biologists will routinely use mathematical models and computational approaches to frame hypotheses." As Cohen (2004) points out, "mathematics can help biologists grasp problems that are otherwise too big (the biosphere) or too small (molecular structure); too slow (macroevolution) or too fast (photosynthesis); too remote in time (early extinctions) or too remote in space (life at extremes on the earth and in space); too complex (the human brain) or too dangerous or unethical (epidemiology of infectious agents)." Biologists also increasingly use computer programs to answer questions, but, without a theoretical understanding of mathematics and programming, we "may misapply the method, miss important observations, misinterpret the results, and derive erroneous biological conclusions" (Pevzner and Shamir 2009, Brent 2004, Cohen 2004, May 2004).

Both scientists and independently thinking citizens need quantitative literacy skills, such as representing and interpreting data in graphs and articulating and critiquing data-based arguments (Speth et al. 2010, Marsteller et al. 2010, Chevalier et al. 2007). These skills, in addition to a basic introduction to mathematical modeling, should be included in introductory and non-majors biology courses and reinforced and expanded throughout the biology curriculum.

One major challenge in this regard is that students often do not see biology as a quantitative science (Spall et al. 2003, Speth et al. 2010, Matthews et al. 2010, Gross 2004, Crow 2004) and are surprised to learn that contemporary biological research often requires high-level mathematics (Hodgson et al. 2005), primarily because biology education at all levels, from elementary school to undergraduate education, tends to emphasize qualitative and descriptive observations used by "naturalists" before technology provided instrumentation to quantify those observations (Crow 2004). In contrast to the physical sciences that focus on principles and reasoning, students in biology often "find themselves focused more on mastering huge arrays of facts" (Bialek and Botstein 2004). Momsen et al. (2010) analyzed course goals and assessments in 77 introductory biology courses from 50 institutions and concluded that that 93% of 9,713 analyzed exam and quiz questions focused on low-level cognitive skills (described in Bloom's Taxonomy as knowledge and comprehension), even though course goals included higher-order cognitive skills (such as analysis, application, synthesis, and evaluation). "The training process for biology tends to attract students who are good at memorization, who work effectively from concrete examples to general principles, who enjoy struggling to understand complicated systems even if they must sometimes reason qualitatively, and who revel in finding and characterizing new details of a system" (Chiel et al. 2010).

Although biology majors are typically required to take one or two semesters of mathematics, Ellison and Dennis (2010) found that 12 of the 50 institutions that produce the majority of students who go on to receive Ph.D.s in the life sciences required no quantitative courses for life science majors. These mathematics courses are usually calculus and occasionally include requirements in statistics or computer programming (Gross 1994). These courses, even when specifically intended for biology majors, often use nonbiology examples and rarely teach mathematics skills essential for modern biology (Thompson et al. 2010). They are typically taught by mathematics faculty who rarely make connections to the biological sciences and often greatly reduce the sophistication and difficulty of quantitative content (Bialek and Botstein 2004). Students rarely use mathematics and physical sciences in their introductory and upper-division biology courses and see the prerequisites more as obstacles to overcome than as useful for future courses and careers in biology (Nelson et al. 2009).

Citing as evidence that most introductory biology textbooks provide no mathematical principles or formulas and only list conclusions rather than revealing the scientific process, Crow (2004) argued that separating mathematics from biology “misrepresents contemporary biology research and … produces generations of biology students who cannot conduct experimental research or interpret research.”

Barriers to Integrating Mathematics into Biology Education:

Faculty cite many reasons for not better integrating mathematics into the biology curriculum, including:

- Students’ negative reactions (“mathematics anxiety”)
- Students’ lack of mathematics mastery
- Necessity of reducing biology content
- Possible negative impacts on students’ learning of biology concepts
- Cultural gap between biologists and the (historically) more quantitative sciences
- Faculty’s lack of mathematics mastery
- Institutional barriers

Instructors may not want to include mathematics in biology courses because students often react negatively and may criticize or be less likely to enroll in biology courses that use math. These negative reactions may be related to the psychological phenomenon of “mathematics anxiety,” defined as “a feeling of tension and anxiety that interferes with the manipulation of numbers and the solving of mathematical problems in a wide variety of ordinary life and academic situations” (Richardson & Suinn 1972).

Mathematics anxiety can cause students to abandon a career in the life sciences or any field requiring mathematical aptitude or analysis (Ashcraft et al. 2009). Dreger and Aiken (1957, in Chiel et al. 2010) first coined the term "mathematics anxiety," but Sheila Tobias popularized the importance of mathematics anxiety for career choices, particularly for young women and minorities, in her influential book *Overcoming Mathematics Anxiety*, first published in 1978. Ashcraft and Moore (2009) summarize psychological literature showing that mathematics anxiety is not correlated to intelligence (as measured by IQ tests), but is correlated to text anxiety and other recognized forms of anxiety. It often results in lower scores on mathematics achievement tests, lower grades in mathematics courses, enrollment in fewer mathematics courses, and avoidance of majors and careers that require mathematics (including physical sciences, engineering, and math). Mathematics anxiety is more common and severe in women and older people and in people with inadequate mathematical background and achievement (Betz 1978). Mathematics anxiety may be relatively common among college students: e.g., 30% of 473 students in Betz (1978), 23% in Ashcraft and Kirk (2001), and 85% of students in Perry (2004).

Instructors can recognize mathematics anxiety and utilize various strategies to reduce it in their students. For example, Tobias (1995) suggests learning mathematics in a low-pressure environment, such as: at home with no competition with classmates or insults; allowing the use of calculators, computers, or a list of formulas; and working in groups. Some instructors, including the developers of the MathBench modules (Thompson et al. 2010, Nelson et al. 2009), attempt to reduce mathematics anxiety in college biology students by providing the opportunity to work at home and use technology, informal language, everyday or biology situations, and self-pacing. Other instructors have students work on quantitative problems in groups (Hodgson et al. 2005, Chiel et al. 2010). Instructors can also provide information on overcoming mathematics anxiety (e.g., Bohrod et al. 2013), and some institutions provide workshops or courses to describe mathematics anxiety, such as those described in Tobias (1995).

One reason often cited by instructors and textbook publishers for not using mathematics in biology courses and textbooks is students' lack of mathematics mastery (Crow 2004). For example, because many introductory biology courses do not require any college mathematics courses, instructors can only utilize high school math. However, it is possible to either use only high school mathematics or to teach mathematics concepts as they are necessary for the course curriculum. Developers of an introductory biology course at Indiana University created a set of biomathematics problems that illustrated the modeling cycle, importance of assumptions, and importance of mathematics in biology while using only secondary-level mathematics (Hodgson et al. 2005). Similarly, the MathBench modules only use concepts up to precalculus (Thompson et al. 2010, Nelson et al. 2009). Several authors recommend re-designing required mathematics courses for biology majors to include specific concepts useful in biology, in addition to calculus (Pevzner & Shamir 2009, NRC 2003b, Gross 2004). Even after students have taken required mathematics courses, biology instructors should reinforce what students learned in mathematics courses and "teach the necessary mathematics as situationally and pedagogically needed" (Hoy 2004).

Some faculty may be concerned that integrating mathematics into biology courses may require reducing the biology content in their courses. A solution to this problem is to put the mathematics or some biology content into online or take-home assignments, as in the online MathBench modules (Thompson et al. 2010, Nelson et al. 2009). As well, other recommendations in the *Vision and Change* (AAAS 2011) and other reports include introducing fewer concepts in greater depth and using learner-centered and inquiry-driven pedagogical strategies (AAAS 2011), which may provide more time and opportunities for practicing quantitative skills. Gross (2004) argues that worries about "leaving out" content implies students are "empty vessels that information can be pumped into [and] that if this information isn't imbibed in your class they'll never be able to get it" (e.g., behaviorism/empiricism as described in Greeno et al. 1996). He adds that if instructors are successful in developing students' capacity for critical thinking and problem-solving, students should "be able to ascertain what knowledge they lack in order to investigate a certain problem and how to learn about that area (or collaborate with an expert in it)."

Instructors may also be concerned that incorporating mathematics may decrease students' learning of biological concepts in the course, possibly due to mathematics anxiety, overload of material, or student focus on mathematics rather than the biological concepts. Madlung et al. (2011) demonstrated that using a quantitative learning tool requiring active calculations had no impact on learning of biological concepts by introductory students and a positive impact on advanced students. Because mathematical concepts are essential to many biological concepts, such as population genetics, Mendelian genetics, population dynamics, incorporating mathematics helps to explain the biological concepts.

Many authors cite a cultural gap (in both faculty and students) between biologists and the (historically) more quantitative sciences, which use different terminology and language and different "ways of knowing" (Chiel et al. 2010, Sung et al. 2003). For example, biological education tends to encourage and attract students who are good at memorization of facts and finding details of systems, while mathematics, physics, and engineering encourage reasoning with abstract principles and finding the right simplifications and abstractions to describe a system (Chiel et al. 2010). NRC (2003a) also recognized a communication barrier between researchers trained in physics and chemistry and researchers trained as biologists, which discourages interdisciplinary research. Some students recognize this cultural gap and the need for interdisciplinary projects and decide to major in two departments, but some schools are discouraging double-majoring by pressuring students to graduate sooner (Gross 2004). In another approach, faculty of multiple departments can team-teach interdisciplinary classes for students of multiple departments. Sung et al. (2003) also recommended that funding programs should encourage interdisciplinary projects by requiring graduate and postdoctoral fellows to find advisors in multiple departments, so that “shared trainees are the catalysts bringing research groups together.”

Another challenge is that some faculty may not feel comfortable teaching advanced courses in mathematical (or quantitative) biology or perhaps even integrating mathematics into biology courses. For example, developing new interdisciplinary courses providing an introduction to biology and chemistry at Harvard University “depended on finding faculty members with personal commitments to the principles of the courses and willingness to work as a team to build the new courses from scratch” (NRC 2009). Institutions can overcome this barrier by encouraging faculty from multiple departments to design and teach courses together. Hoy (2004) and Brewer and Gross (2003) also recommend offering workshops and short courses on computational and mathematical biology for current faculty and researchers.

Finally, some authors cite institutional barriers to integrating mathematics into biology curricula. For example, students often can't take more mathematics or quantitative biology courses because they are under pressure to finish quickly (Gross 1994), and departments often are unable to require students to take additional courses because the number of units is limited (Chiel et al. 2010). Faculty may not have time to revise or develop new courses, and departments may not give teaching credit for team-taught and/or interdisciplinary courses, even though team-teaching often requires more time than teaching a course solo (Chiel et al. 2010, Sung et al. 2003). NRC (2003a) recommends starting by integrating modules (self-contained sets of material on a specific topic that can be inserted into different types of pre-existing courses) on quantitative skills into existing biology courses. Many modules on quantitative skills are now available online (listed in Marsteller et al. 2010). Instructors must also find support from their departments and institutions for developing new courses and team-teaching interdisciplinary courses (Hoy 2004). For example, when Harvard University developed new courses giving an interdisciplinary introduction to biology and chemistry, Harvard provided funds for a one-year curriculum development effort, lab renovations, lower teaching fellow–student ratios, equipment, and development of teaching materials (NRC 2009). Just as importantly, individual departments at Harvard agreed to count these interdisciplinary courses toward departmental teaching expectations.

Strategies for Integrating Mathematics into Biology Education:

National calls to action (AAAS 2011, NRC 2003a) and many authors describe a variety of strategies for improving quantitative education in biology curricula, but all emphasize that reforms must be led by biologists and biology departments, with support from institutions, other disciplines, and funding and government agencies such as the National Science Foundation (NSF). Hoy (2004) emphasizes that leadership by mathematicians and lack of institutional support were responsible for the failure of the visionary curriculum reform called “Mathematics Across the Curriculum” that began at Dartmouth University with NSF support and developed curricula and textbooks for integration of mathematics into physical and biological sciences, humanities, and the social sciences. AAAS (2009) points out that department-wide change often happen when biology departments hire a biologist with an education specialty, who can collaborate with other science and education faculty to address curricular efforts. Many institutions, such as the University of Delaware, have implemented several of these strategies (Usher et al. 2010).

In biology education literature, strategies for integrating mathematics into undergraduate biology curricula include (in decreasing order ease of use from practical in-classroom suggestions to institutional changes):

- Integrate quantitative concepts into biology courses
- Develop modules that can be integrated into existing courses
- Explicitly link biology and mathematics courses
- Include quantitative skills in undergraduate research
- Integrate biological examples into mathematics courses
- Redesign quantitative classes for biology majors to include more than just calculus
- Develop new “hybrid” interdisciplinary courses
- Offer new interdisciplinary majors & minors
- Offer professional development workshops for faculty

- Use evidence-based and learner-centered pedagogical strategies

Many authors recommend that quantitative skills should be introduced in introductory biology classes and reinforced and expanded throughout the biology curriculum (NRC 2003a, Crow 2004, Chevalier et al 2007, NRC 2009, Speth et al. 2010, and many others). Thompson et al. (2010) argue that current special courses and curricula focused on quantitative biology benefit only the strongest students and instead they recommend infusing mathematics throughout the entire undergraduate biology curriculum for all students, starting with introductory biology courses. Hoy (2004) emphasizes that "biology faculty should instruct their students in the necessary mathematics as situationally and pedagogically needed," but "this does NOT mean that biologists should teach the mathematics basics . . . but that they should reinforce what their mathematics colleagues had taught their students earlier." Ellison and Dennis (2010) emphasize that, in addition to taking calculus courses, students must use calculus in courses in ecology, resource management, and environmental science in order to be prepared to test hypotheses, model data, and forecast future environmental conditions.

Several papers describe how instructors integrate quantitative skills into introductory courses at their institutions, with a range of emphases (Speth et al. 2010, Hodgson et al. 2005, Chevalier et al. 2007, Usher et al. 2010, Nelson et al. 2009, Thompson et al. 2010). Most of these courses emphasize quantitative skills primarily in laboratory exercises and require only high school math. For example, Hodgson et al. (2005) describe an introductory biology course at Indiana University in which they explicitly introduce mathematical modeling and the modeling cycle in lectures and require students during recitation/discussion sections to work in groups on biomathematics problems that require only high school math, use authentic biological contexts, and encourage students to identify assumptions. Speth et al. (2010) describe incorporating quantitative literacy, which they define as the “ability to interpret data and to reason with numbers within ‘real-life’ situations,” throughout an introductory biology course at Michigan State University. Usher et al. (2010) describe how they hired mathematics undergraduates and graduate students to help design activities requiring quantitative approaches as part of laboratory exercises. Chevalier et al. (2007) describe how integrating hypothesis formulation, experimental design, descriptive and hypothesis-testing statistics, and building graphs into laboratory exercises in an introductory biology course at Missouri Western State University resulted in significant improvement in quantitative skills and retention for at least two years through other biology classes that did not have a similar focus on quantitative skills.

NRC (2003a) recommends starting to improve quantitative skills in biology curricula by integrating modules (self-contained sets of material on specific topics that can be inserted into various pre-existing courses) on quantitative skills into existing biology courses. While many modules on quantitative skills are available online (Marsteller et al. 2010), one set of modules that is extremely easy to use in introductory and other courses is the MathBench modules developed at the University of Maryland (Nelson et al. 2009, Thompson et al. 2010). These are interactive web-based modules that introduce the mathematical underpinnings of introductory biology and basic statistics and graphing, using informal style, engaging storylines, intelligent feedback and scaffolding that support students to reach the correct answer (Wells 1999), and learner control (self-pacing) to reduce mathematics anxiety. The modules focus on 10 mathematical meta-skills requiring only pre-calculus as a pre-requisite, such as parsing verbal descriptions into mathematical equations, using graphs and equations, converting units, using common statistic terms and tests (t-test, chi-square), and understanding the structure and differences between mathematical models. Each module includes an online quiz for assessment. All 36 modules are freely available online at www.mathbench.umd.edu.

Biology faculty can also link introductory biology courses to appropriate mathematics courses (Hodgson et al. 2005), as seen in Florida International University's "Quantifying Biology in the Classroom" (QBIC) program for research-oriented biology majors (http://qbic.fiu.edu).

Biology faculty can explicitly target research-oriented undergraduates by encouraging undergraduates to participate in scientific research, particularly independent research, and including practice of quantitative skills in research experiences. NRC (2009) recommends that biology faculty should encourage students to "pursue independent research as early as is practical in their education." Jones et al. (2009) find that participation in undergraduate research is positively associated with the likelihood of obtaining a baccalaureate degree, persisting in biology, and performing well in biology (particularly among Hispanic Americans and African Americans), perhaps because students participating in undergraduate research "gain academic, practical, and professional skills necessary to develop a positive identity as a scientist and continue in a science career" and "greater understanding, confidence, and interest in science careers." Gross (2004) and Crow (2004) particularly recommend student participation in research that includes data collection, statistical analysis, and modeling.

Several authors recommend integrating biological examples into mathematics classes for life science majors (NRC 2003a, NRC 2009 and others). Mathematics courses, even those specifically intended for biology majors, often focus on physical science examples rather than biological examples (Thompson et al. 2010). For example, Usher et al. (2010) describes how the University of Delaware created a "life science interest" section that is conceptually identical to other nonbio-calculus sections, but draws models and examples heavily from life sciences. During three years when students in both versions of the class took the same final exam, biology majors performed at least as well as (and often better than) other science majors. This approach requires mathematics and biology faculty to work together to design and/or teach courses for life science students. This recommendation is founded in the learning theory of situated cognition, in which education researchers advocate teaching abstractions with specific examples and situations from everyday life or scientific research, including examples that illustrate the economic, sociological, technological, and political roles of science in the modern world (Brown et al. 1989, Lemke 2001). For example, Brown et al. (1989) argue that "learning methods that are embedded in authentic situations are not merely useful; they are essential." They also point out that practitioners must solve problems that are much less defined (more messy) than the usual problems in school textbooks and exams are. Marsteller et al. (2010) provide contact information for several current quantitative courses using biological examples.

To focus on the quantitative and mathematical skills most essential to the life sciences, faculty may need to change quantitative requirements and/or re-design mathematics and programming courses (Pevzner and Shamir 2009, NRC 2003a, Hoy 2004, Gross 2004). Although most biology majors are required to take two semesters of mathematics, the main difference between most mathematics courses for physical science and engineering majors and for life science majors is that the courses for life science majors are less difficult and less sophisticated rather than specifically designed for life science majors (Bialek and Botstein 2004). The BIO2010 report (NRC 2003b) concludes that mathematics courses required for biology majors rarely teach mathematics skills essential for modern biology and recommends that such courses should include aspects of probability, statistics, discrete models, linear algebra, calculus and differential equations, modeling, and programming. Hoy (2004) argues that courses should "teach [mathematics], without the formal rigor necessary for a practicing mathematician, to biologists . . . that would enable them to articulate biological problems in a form that would lead to congenial collaborations with mathematicians who understand [math] deeply." Robeva and Laubenbacher (2009) recommend teaching algebraic models because they are valuable in biology and do not require previous knowledge of calculus, so they can "provide a quick path to mathematical modeling for students and researchers in the life sciences." Similarly, Hoy (2004) points out that discrete mathematics is both highly relevant for today's life sciences and more user-friendly to biologists than calculus. Ellison and Dennis (2010) recommend that both ecologists and resource managers should pass two semesters of standard calculus and a post-calculus statistics course in college and a two semester, post-calculus sequence in probability and mathematical statistics in the first two years of graduate school, in addition to using calculus and statistics in ecology and environmental science classes.

Several institutions have re-designed required mathematics courses to better reflect these recommendations. For example, Gross (2004) re-designed the mathematics courses required for life science majors at University of Tennessee to include "descriptive statistics, matrix algebra (including eigenvalues and eigenvectors), discrete modeling, and probability, as well as the concept of an algorithm, through computer-based projects requiring the use of an appropriate mathematical software package" (Matlab). Usher et al. (2010) describe how they worked with mathematics faculty to require biology majors to take mathematics for physical science majors rather than business-oriented mathematics and re-ordered the concepts in the three-semester introductory mathematics sequence to fit concepts most essential to life science majors into the first semester, which was the only one required for life science majors.

Many authors also recommend that biology majors should have at least some experience in computer programming (including teaching about computer algorithms and constructing simple simulations) in higher-level languages such as Matlab and Mathematica (NRC 2003a). NRC (2003a) specifically recommends that all life science majors should be able to "carry out simulations of physiological, ecological, and evolutionary processes" and use "computers to acquire and process data, carry out statistical characterization and tests of data, and graphically display data in a variety of representations." Similarly, AAAS (2011) recommends that "all students should understand how mathematics and computational tools describe living systems and be able to implement computational algorithms for models. Pevzner and Shamir (2009) recommend implementing and requiring computational biology courses that present the concepts, ideas, and real-life impacts of computational biology, but do not require knowledge of computer programming. They recommend focusing on biological questions (such as "Did our ancestors interbreed with Neanderthals?") and following with the computational ideas used to answer them. They point out that most bioinformatics courses at leading universities are not ideally suited for biology students. These authors propose the use of a variety of programming languages, including Mathematica (Chiel et al. 2010, Hoy 2004, Bialek and Botstein 2004, NRC 2003a), Matlab (Hoy 2004, Bialek and Botstein 2004, NRC 2003a), Perl and C (NRC 2003a), and Python (Matthews et al. 2010). More recently, quantitative biology courses may use the open-source language R, for which many packages accomplishing a wide variety of tasks are available.

Several universities have developed “hybrid” interdisciplinary courses as either introductory or upper-division classes in biology. Bialek and Botstein (2004) advocate an introductory course for future biology researchers that integrates biology, math, physics, chemistry, and computer programming and which would encourage future interdisciplinary collaborations among instructors and students. Gross (2004) critiques recommendations for such interdisciplinary courses because they often lack sufficient institutional support and rely completely on one enthusiastic instructor and because it’s difficult to identify and target future researchers. Matthews et al. (2010) implemented such a course, specifically based on the BIO2010 (NRC 2003a) recommendations, at University of Queensland, which they called “Theory and Practice in Science” and used as a gateway course highly recommended for all science majors. They describe their course as “interdisciplinary in nature, not only demonstrating the mathematical foundations that underpin a range of science disciplines but also highlighting links between different science disciplines.” Instructors from both mathematics and life sciences led interactive lectures in which “students solved context-based mathematical problems during classes and were encouraged to work with their peers.” Tutors from mathematics and science team-taught tutorials and computer practicals using the computer language Python. The course focused on real-world themes in life sciences, such as heart disease and climate change, and introduced concepts in mathematics and science necessary for discussions. They found that, at the end of the course, students agreed that mathematics is important to biology and that they were enthusiastic about math, but students did not hold positive perceptions of computer programming and were not motivated to take other quantitative courses.

Chiel et al. (2010) developed a hybrid upper-level course for students majoring in biology, engineering, physical sciences, and mathematics at Case Western Reserve University. The first half of the semester focuses on developing quantitative skills (such as nonlinear dynamics and programming in Mathematica) using an interactive textbook and benchmark problems. Instructors reduce scaffolding (instructor assistance and guidance, Wells 1999) over time and emphasize Socratic questioning, which the authors define as ”asking students leading questions and guiding student to grasp the correct answer, rather than stating the answer.” In the second half of the semester, students reconstruct and extend a model from a technical paper, write a paper, and present their work to the class orally. Throughout the course, students work in the same randomly chosen teams of two students.

In my opinion, the design of this course exemplifies recommendations of several major learning theories and the *Vision and Change* report (AAAS 2011). By reducing scaffolding through time in the first half of the course and emphasizing in the second half of the course that students are experts on their models and instructors are only advisors, instructors create a true “community of practice” in which students increase their participation through time, which Lave (1991) calls “legitimate peripheral participation.” Because students work in teams, with help from their tablemates and instructors, they are working in their “zone of proximal development,” which Vygotsky (1978, in Wells 1999) defines as “distance between actual developmental level as determined by independent problem solving and level of potential development as determined through problem solving under adult guidance or in collaboration with more capable peers.” The instructors also emphasize Socratic questioning, which Tharp and Gaillimore (1988) call the only form of teaching to produce understanding. The final project is an example of a project that requires integration of many ideas (as in Darling-Hammond et al. 2001) and full open inquiry (NRC 1996, Minner et al. 2010). The course also follows the recommendations of AAAS (2009) by being student-centered (including cooperative, active, and inquiry-driven learning), assessment-centered (with benchmark problems), by emphasizing communication (with a final presentation and discussions throughout the course), and by using problem-based learning (in final project).

To encourage interdisciplinary education without the necessity of double-majoring, several institutions have developed new programs and/or majors in quantitative biology. Marsteller et al. (2010) and Usher et al. (2010) summarize the results of a survey of existing interdisciplinary math/biology majors. Although they found many programs in bioinformatics and computational biology that developed skills in statistics and computer science, they found only 11 “truly interdisciplinary majors” (called mathematical, systems, or quantitative biology) that focused on developing skills to be used in producing predictive mathematical models of biological systems. Programs in mathematics departments required more mathematics courses and programs in biology departments required more biology courses, but few programs required more than four semesters of chemistry and two semesters of physics (which is standard for biology majors) and an introductory-level computer sciences course. Almost all programs required a capstone course, seminar, and/or an undergraduate research experience. A few programs developed new courses for their curricula.

Based on this survey, the University of Delaware developed a Quantitative Biology major, which required two more courses of mathematics than other biology majors, investigative lab courses based on “discovery learning,” a new seminar focused on cutting-edge biology research problems in which mathematics plays a central role, and a project-based introductory course to systems biology (Usher et al. 2010). Students are encouraged to participate in undergraduate research, particularly in pairs of mathematics and biology students. Although the major is housed in the Mathematics department, it is listed on the Biological Sciences department website and catalog and its courses count for Biology faculty’s expected teaching workload. The program includes a steering committee of faculty from both mathematics and biology departments and an external advisory board of researchers from medical centers and industry. The authors report that, two years after the program was approved, there were 20 students majoring in Quantitative Biology, including five students who applied to the university specifically for the Quantitative Biology major.

In order for faculty, researchers, and managers to maintain and extend their own quantitative skills, Hoy (2004) and Brewer and Gross (2003) recommend offering workshops and short courses in computational and mathematical techniques, perhaps at meetings of professional societies

Many authors recommend that courses integrating mathematics and biology should also make use of learner-centered and inquiry-driven pedagogical strategies which are supported by evidence in learning and education research and by learning theories. These engaging teaching strategies are particularly important when teaching mathematics to biology students who often dislike or are anxious about math. These strategies include:

- Using real-world examples and themes (as in case study teaching) and current biological research (AAAS 2011, NRC 2003a, Matthews et al. 2010, Chiel et al. 2010, Hoy 2004, Speth et al. 2010). This is predicted by the theory of situated cognition (Brown et al. 1989);
- Teaching fewer concepts in greater depth (AAAS 2011), based on the theory of constructivism (NRC 2003b) first developed by Jean Piaget;
- Active learning (AAAS 2011, NRC 2003a, Speth et al. 2010, Chiel et al. 2010, Hoy 2004), also based on the theory of constructivism (NRC 2003b);
- Inquiry-based learning in which students "engage in many of the same activities and thinking processes as scientists" (AAAS 2011, NRC 2000, NRC 2003a, Speth et al. 2010, Derting and Ebert-May 2010), also based on the theory of constructivism (NRC 2003b, Minner et al. 2010);
- Project-based learning (NRC 2003a), also based on the theory of constructivism; and
- Cooperative (or collaborative/group) learning (AAAS 2011, Chiel et al. 2010, Hodgson et al. 2005), based on sociocultural theory (Wells 1999, Brown et al. 1989) first developed by Lev Vygotsky.

Introduction to Experimental Study:

Integrating mathematics and biology in undergraduate courses requires demonstrating math's use for and importance to biological concepts and research, minimizing mathematics anxiety, and using learner-centered pedagogical strategies.

Because many students are anxious about and/or dislike math, it is particularly important to use pedagogical strategies with characteristics such as those recommended in AAAS (2011): interactive (with high levels of student-student and student-faculty interaction); cooperative and collaborative (with students working as teams to accomplish a shared goal); inquiry-driven (reflecting diverse aspects of scientific inquiry, including data interpretation, argumentation, and peer review); and relevant (connecting course subject matter to topics students find relevant). This focus on relevance is also reflected in the AAAS (2011) six core competencies and disciplinary practices that include the ability to apply the process of science and the ability to understand the relationship between science and society. The Next Generation Science Standards for K-12 also emphasize inquiry in the Science and Engineering Practices dimension and relevance in the core idea of "the influence of engineering, technology, and science on society and the natural world" (NGSS Lead States 2013).

One of the best strategies for demonstrating the importance of mathematics and science to society and to each other, with a long history in classrooms and strong supporting evidence in educational research, is the use of case studies, which Herreid (2011) defines as realistic or true "stories with an educational message [that] put learning into a context that is memorable." Despite the many modes used with case studies (e.g., lecture, whole-class discussions, small groups, individuals), faculty who have used case studies reported that students learning via case study-based instruction demonstrated stronger critical thinking skills, were able to make connections across multiple content areas, developed a deeper understanding of concepts, were better able to view an issue from multiple perspectives, and had a better grasp of the practical applications of core course concepts (Yadav et al.

2007). Chaplin (2009) reports that using case study-based instruction in her introductory biology course increased students' ability to answer challenging application and analysis questions and improved students' performance on exams. In an introductory microbiology course, 64% of 340 students reported that they thought case studies were very helpful, because case studies helped them to learn/think about/apply course concepts, case studies allowed them to see the real-world relevance of course concepts, and the case studies made the course concepts more interesting or more engaging (Smith et al. 2005).

As a first step to providing better training in quantitative skills necessary for future biologists and educated citizens, NRC (2003a) recommends integrating modules (self-contained sets of material on specific topics that can be inserted into various different types of pre-existing courses) on quantitative skills into existing biology courses.

Here I present and analyze a computer inquiry module using case studies from ecological research literature to introduce structured population models (Caswell 2001), one of the most commonly-used types of ecological models, and their application in conservation biology and ecology. I tested and refined the module in an undergraduate biology course in 2012 and 2013. I examine whether the module accomplished its goals of students gaining a better appreciation of the importance and uses of mathematics in ecology and conservation and demonstrating their ability to apply structured population models to assessment and management of populations in a variety of contexts. In this lesson, students:

- Participated in an interactive lecture using a case study to introduce the concepts of structured population models and the mechanics of representing population structures in matrices

- Read published research papers that apply structured population models to specific populations and conservation questions
- Worked through case studies in groups to manipulate population transition matrices on computers using the MS Excel add-on PopTools, and
- Briefly presented the model with their own research question to the class

Methods and Materials:

Description of Module

The module includes one interactive lecture (105 min in 2012, 70 min in 2013) followed by a 2-3 hr cooperative computer inquiry activity based on a reading assignment. The lecture was built around a Powerpoint presentation that covered the concepts of structured populations (i.e. individuals differ in age, size, or other characteristics) and how population structures can be expressed in matrices. These concepts were embedded in a case study demonstrating how such models are used in conservation biology, using interactive elements recommended by AAAS (2011). During the activity, groups discussed structured population models in several papers about different organisms, applied the concepts from the lecture to their model, asked and answered their own research question using the model, and presented their model and research to the class in brief oral presentations.

The lecture illustrated the application of the scientific process in the case study, which was based on recent scientific research and a real-world conservation problem and included fieldwork, labwork, and mathematical modelling. The lecture specifically discussed the definition of a model and the process of developing a mathematical model, including specifying the research question, identifying appropriate assumptions, and developing a conceptual model (life cycle diagram, in this case, as described in model-based learning theory such as Gobert and Buckley 2000) before adding numbers. Interactive elements of the lecture included "Think-Pair-Share" discussions of conceptual questions (e.g., "What is a model?"), problems (e.g., translating an example life cycle diagram to a transition matrix and vice versa), and brainstorming (e.g., anthropogenic threats to albatrosses). The lecture included:

- introduction to the case study and its need for mathematical modeling
- introduction to models and mathematical models in ecology
- introduction to structured population models, including
    - simpler exponential and logistic growth models
    - life cycle diagrams and transition matrices for populations
    - estimation of vital rates for a population
    - assumptions of structured population models
- application of structured population models and their analysis to the case study, including
    - population growth rate (dominant eigenvalue – λ)
    - population projection graphs
    - reproductive value distribution
    - elasticity
    - stable stage distribution

The lecture interpreted these concepts in an ecological context and provided, but did not go into detail on, the actual mathematical calculations used to derive them. Instead, the lecture explained the use of PopTools (Hood 2010), which was used in the accompanying lab. PopTools is a free menu-based add-on to Microsoft Excel which provides the easy calculation of the analyses described above as well as many others.

In 2012, the case study used in lecture was my ecological research on the decline and possible restoration of black-lipped pearl oysters (*Pinctada margaritifera*) in the Northwestern Hawaiian Islands. In 2013, the lecture used the story of lead poisoning of Laysan Albatrosses at Midway Atoll (Finkelstein et al. 2003, 2009); a revised version is available with all materials and lesson plans at http://www.tiny.cc/ecolmodelsmodule. Students were asked to read the albatross model paper (Finkelstein et al. 2009) before the 2013 lecture, but only a few students indicated that they had read it.

Before the activity, each student chose a particular organism for which a structured population model paper was available. Although the instructors asked for groups of three or four students, a few groups had only one or up to five students. The instructor had pre-selected six different papers, each including a structured population model for an organism from the coral reef, mangrove, and seagrass ecosystems and made these available to students through the online course management system. Topics were: mangroves (Clarke 1995), turtles (Crouse et al. 1987), corals (Hughes & Tanner 2000), sharks (Robbins et al. 2006), manatees (Runge et al. 2004), and fish (Wielgus et al. 2007). Students were instructed to read the paper before the lab and to be prepared to answer a quiz on the paper’s research question, life cycle diagram, methods of estimating vital rates, and conclusions.

During the lab, students who had all read the same paper formed groups with access to at least one PC laptop with Microsoft Excel and the PopTools add-on per group.

The instructor quickly reviewed the ecological interpretation and use of PopTools for population growth rate, elasticity, stable stage distribution, and reproductive value. Groups, with the facilitation of instructors, then drew on their papers to extract the research questions, identify life history stages, list vital rates, make life cycle diagrams, and inserted appropriate vital rates into a transition matrix. They then used PopTools to analyze the transition matrix to answer the following questions:

- Is the population growing (after many years with these vital rates)?
- Which vital rate(s) should managers focus monitoring and conservation effort on?
- Which stage(s) should managers focus monitoring and conservation effort on?
- Which stage(s) contain most of the population (after many years with these vital rates)?

Each group then developed its own research question (generally focusing on simulating possible management actions) and used PopTools to answer it. Finally, each group briefly presented its population, life cycle, and research question to the class with a hand-drawn poster.

About three weeks after the module ended, all students answered a set of multiple-choice and free-response questions about the module on their lab practical exam. These questions are provided in the appendix.

Although this module was originally designed for an upper-division ecology course focused on particular ecosystems, instructors can easily adapt it for other upper-division ecology courses, mathematics or hybrid courses for biology majors, or perhaps even ambitious lower-division courses simply by selecting more appropriate research papers for students to read in the inquiry lab from the huge variety of research using structured models to assess populations.

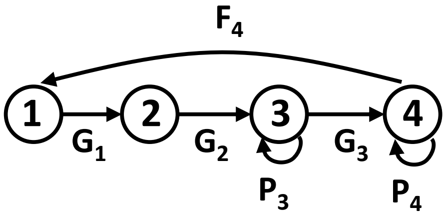
Description of Mathematical Model: Structured Population Models

The life history of most organisms can be divided into several classes (or groups) based on size, age, developmental stage, sex, or another variable. Incorporating this structure into a mathematical model often makes the model more accurate and precise and allows the examination of specific classes for any purpose, such as predicting the impact to a turtle population of protecting eggs on a beach. Population biologists generally depict this life history in a life cycle diagram (Figure 1a). For example, the life cycle of a fish may be divided into larvae, juveniles, sub-adults, and adults or year 1, 2, 3, and 4. Individuals in a particular class may grow into another class (e.g., G_3), persist in the same class (e.g., P_3), and/or reproduce (e.g., F_4).

This life history is often depicted mathematically as a transition matrix, which is a mathematical tool for describing transitions between classes and is basically a series of linear equations organized to simplify mathematical operations such as multiplication. As Figure 1b shows, we can determine the number of individuals in each class at time t+1 by multiplying the transition matrix by the number of individuals in each class at time t (or the population vector). Once scientists have used field or lab work to determine the actual numeric values in the transition matrix, we can analyze it in a variety of ways to learn about the population and how to best manage it for conservation purposes (Table 1). In the Protecting Populations module, students use the easy Microsoft Excel add-on PopTools to calculate population growth rate, population projection, reproductive value, stable stage distribution, and elasticity.

The transition matrix, together with tools for analyzing it, is known as a structured population model because we are not assuming that all individuals in the population are identical, as in standard exponential and logistic population growth models. Structured population models are described in great depth in Caswell (2001) and in most mathematical ecology texts (e.g., Gotelli 2001), although they are sometimes called matrix population models.

(a)



(b)

$$\begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \end{bmatrix}_{t+1} \overset{\text{TO}}{=} \overset{\text{FROM}}{\begin{array}{c|cccc} & 1 & 2 & 3 & 4 \\ \hline 1 & 0 & 0 & 0 & F_4 \\ 2 & G_1 & 0 & 0 & 0 \\ 3 & 0 & G_2 & P_3 & 0 \\ 4 & 0 & 0 & G_3 & P_4 \end{array}} \begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \end{bmatrix}_{t}$$

Figure 1: (a) Example of a life cycle diagram showing some of the possible life history transitions between each of four demographic stages (1 = larvae, 2 = juvenile, 3 = sub-adult, 4 = adult) and (b) corresponding transition matrix projection.

Table 1: Key terms of structured population models and their biological and mathematical definitions from Gotelli (2001).

Term	**Biological Definition**	**Mathematical Definition**
Transition matrix	Describes all the ways that individuals of a given class can transition to (or contribute to) other possible states over the course of a single time-step.	
Population vector	Number of individuals in each class at a particular time.	
Vital rate	Rate of transition or contribution from one class to another, such as growth rate, survival rate, or fertility rate. (e.g., G_3 is the number of individuals in class 4 in the next time period that will be contributed by each individual in class 3 at the current time).	Part of or the complete element in transition matrix (e.g., G_3)
Population growth rate (λ)	Often called asymptotic, ultimate, or long-term population growth rate.	Dominant eigenvalue of transition matrix. Also, $\lambda = e^r$, where r is the instantaneous growth rate used in a continuous model. If $0<\lambda<1$, population is declining. If $\lambda>1$, population is growing.
Population projection graph	Graph of number of individuals in population over a time period.	Graph of results of matrix multiplication of transition matrix by population vector at various times as in Figure 1b
Reprod-uctive value	Relative contribution to future population growth an individual currently in a particular class is expected to make.	Dominant left eigenvector of transition matrix
Stable stage distribution	Constant proportion of individuals in each class which population reaches over time with constant transition matrix and vital rates.	Dominant right eigenvector of transition matrix
Elasticity	Effect that a proportional change in each vital rate or matrix element has on proportional population growth rate λ. Sensitivity scaled to vital rate and population growth rate in order to compare between vital rates	$\frac{\partial \ln \lambda}{\partial \ln a_{ij}} = \frac{a_{ij}}{\lambda}\frac{\partial \lambda}{\partial a_{ij}} = \frac{a_{ij}}{\lambda}\left(\frac{\boldsymbol{v}_i \boldsymbol{w}_j}{\langle \boldsymbol{v}, \boldsymbol{w}\rangle}\right)$

Setting

This module was taught and evaluated during the winter 2012 and 2013 quarters in an upper-division ecology course at University of California, Santa Cruz (UCSC). The course was BIOE 163: "Ecology of Reefs, Mangroves, and Seagrasses," led by Dr. Donald Potts with the help of a teaching assistant. In 2012, the course, with 61 students, included two 105-min lectures per week and three sections of a 2 hr discussion section/lab. In 2013, with 26 students, the course included three 70 min lectures and two sections of a 3 hr lab per week.

Characteristics of students participating in the module were derived from the course rosters and surveys distributed to the students. The course is an elective for Marine Biology, Ecology and Evolution, Biology, Plant Science, and Environmental Studies/Biology majors at UCSC, so most students were in those majors. The largest major was Marine Biology (57.0% of students in 2012 and 2013). Most students were seniors (67.5%) or juniors (29.0%). 57% of students were female. The most recent mathematics courses for most students in the courses were the second quarter of Calculus with Applications (46% of survey respondents) and Biostatistics (30%), although a few students had completed or were completing the Calculus for Science, Engineering, and Mathematics Majors series. When asked what career they planned to pursue, 53% of 48 survey respondents answered research biologist or university professor, while most of the others answered "I don't know."

I was instructor of the lecture and lab in this module in both years and developer of the module. I had previously served as instructor and teaching assistant for the course, but was not involved in any other part of the course in 2012 and 2013. I developed the module through five iterations of the course from 2009 to 2013, with the analysis described here for only the last two iterations.

The UCSC Office of Research Compliance found this research exempt from Institutional Research Board review (IRB protocol #HS1201814).

Study Design

Formative assessment (i.e., “ongoing assessments designed to make students’ thinking visible to both teachers and students”, NRC 2003a) of the module included interaction with students during lecture and observation and facilitation of the groups during the lab. Summative assessment (i.e., at the end of the module and course) included observation and video recording of the presentations, the sketched posters groups used in presentations, lab worksheets, and multiple-choice and free-response questions included in the class’ lab practical exam 3 weeks after the module ended. Only lab worksheets and exam answers were included in course grades.

Students also volunteered to participate in online pre-module and post-module surveys and in-person post-module interviews by the instructor/author. These surveys and interviews focused on students’ previous experiences and goals in biology and math, their attitudes toward mathematics and its applications in biology, ecology, and conservation biology, and their understanding of the concepts and skills included in the module.

30 students (of a class of 61) in 2012 and 18 students (of a class of 26) in 2013 responded to the pre-survey, post-survey, or both. I combined responses from 2012 and 2013 for a total of 39 pre-surveys and 33 post-surveys (including 24 individuals who submitted both surveys). Students occasionally skipped questions, so the number of answers for each question may be smaller. All 61 students in 2012 and 26 students in 2013 answered the questions on the exam. Interview subjects included five female and three

male students in 2012 and two males in 2013. Interview subjects were selected for a wide range of responses from the students who responded to the pre-survey.

Students answered the pre-survey in the week before the module started and the post-survey within a month after the module ended. The surveys were online, so students could have used other resources or discussed questions with others, but they did not receive course credit for taking the survey. The only external motivation was the chance to win a gift certificate of $10 to the campus bookstore in random drawings of pre-surveys and post-surveys.

All students enrolled in the course answered the exam questions three weeks to a month after the module. Because these questions were part of an exam that counted for a large part of their final course grade, most students studied, but they were not able to use outside resources or consult with others during the exam.

Perception of Importance of Mathematics in Biology

Both pre-module and post-module surveys asked students to select the statement that mostly closely represents their attitude toward mathematics (on a Likert 5-point scale), based on a survey described in Thompson et al. (2010):

- I hate mathematics and try to avoid it
- I don’t like math, but I can cope with it
- I don’t care about mathematics one way or another
- I like math, but I don’t seek it out
- I like mathematics and enjoy having coursework that includes math

Pre-surveys and post-surveys asked students to select the statement that best describes their attitude regarding the relationship between mathematics and biology, ecology, and conservation biology (on a Likert 5-point scale), based on a survey described in Thompson et al. (2010):

- Mathematics is not relevant to biology (ecology, conservation biology).
- Mathematics can be useful in biology, but it's not really necessary.
- Mathematics is helpful in biology.
- Mathematics is essential in biology if you want to do cutting-edge work.
- Mathematics is essential for doing any biology, cutting-edge or not.

Pre-surveys (in 2013 only) and post-surveys (both years) asked students whether they would take a course on quantitative or mathematical ecology. Open-ended questions on the surveys subsequently asked students to further describe their mathematics skills and comfort level and to expand on whether they feel mathematics is important to biology, ecology, and conservation biology.

Interviews also provided a few students with the opportunity to further discuss their previous experiences with using mathematics in biology, their understanding of the importance of mathematics in biology, and their view of this module.

Learning Key Content

Questions on the concepts and skills of structured population models were similar in the pre-module survey, post-module survey, interviews, and exam (Appendix), but based on different but parallel data. Students were provided data resources, including the transition matrix and graphs of population projections, elasticities of vital rates, reproductive value distribution, and stable stage distribution, for a given population based on a research paper the students had not previously seen. The models in surveys and exam resembled models students studied in lecture and lab, but were based on research papers students never saw focused on different organisms (mangroves in pre-survey, turtles in post-survey, and invasive Caribbean lionfish in exam). Questions asked students to interpret and specify which form of data they used to answer questions about: (1) whether the population is growing (based on population growth rate or population projection graph); (2) which life history stage (reproductive value) and vital rate (elasticity) wildlife managers should protect; and (3) which stages reproduce or in which individuals can remain for more than one time period (stage transition matrix). In interviews, students were asked to think aloud as they answered these same questions and were occasionally prompted by additional verbal questions.

Data Analysis

I analyzed responses to the perception questions on the surveys using two-way contingency tables and Pearson chi-square tests to determine if the distributions of responses differed between the pre-survey and post-survey for each question.

I analyzed the number of correct responses to the five parallel questions included on all three assessments using a two-way ANOVA with assessment type, year, and interaction of assessment type and year. I conducted this analysis separately for 22 students who submitted answers to all three assessments and for all students in the course (number of samples varies between assessment types). If the interaction was not found to be statistically significant, but assessment type was, I used Tukey's HSD post hoc test to determine which assessment types had statistically significantly different scores. We also determined Cohen's d effect size for repeated measures (Dunlap et al. 1996) between the pre-test and post-test.

I analyzed responses to the content questions using two-way contingency tables and Pearson chi-square tests to determine if the distributions of responses were different between the pre-survey, post-survey, and exam for each question.

Although using only responses of students who responded to both pre- and post-surveys provides the most statistical power, I did not ask students whether they were interested in taking a quantitative ecology course on the pre-survey in 2012 and I had far more responses on the exam than on the surveys for content questions. Thus, I used all responses for the quantitative ecology course question and content questions and only paired responses for the four perception questions about students' attitudes towards mathematics and the importance of mathematics to biology, ecology, and conservation biology.

I determined whether students who took the Protecting Populations module in BIOE 163 were disproportionately represented among the students who were later enrolled in BIOE 148: Quantitative Ecology in winters 2010 and 2012 using Fisher's Exact test. I used only students majoring in Marine Biology, Ecology and Evolutionary Biology, and Environmental Studies/Ecology and Evolutionary Biology, since 100% of BIOE 148 students and 83% of BIOE 163 students were enrolled in these majors. Enrollment lists and numbers of majors were provided by the UCSC Division of Physical and Biological Sciences (Durcan and Gilovich, pers. comm.).

All statistical analyses except Fisher's exact test were conducted using Systat 13 (Systat Software, San Jose, CA). Fisher's exact test was conducted using the programming language R (R Core Team 2012).

Results:

Analysis of answers to multiple-choice questions on the surveys and the exam revealed quantitative patterns across all students in the course, while answers to open-ended survey questions and interviews suggested probable explanations of these quantitative patterns. Interviews also elicited alternative conceptions, defined as “student understandings of scientific concepts that are not aligned with the current understanding of scientists” (Tanner and Allen 2005), that persisted after the module.

Here I present the results in two major categories: (1) perception of importance of mathematics in biology; and (2) learning key content.

Perception of Importance of Mathematics in Biology

In pre- and post-module surveys, students were asked which statement most closely represented their attitude toward mathematics and the importance of mathematics to biology, ecology, and conservation biology.

Although no students reported that they “hate and try to avoid” math, 37.5% of students reported that they “don’t like, but can cope with” mathematics in both the pre-module and post-module surveys. The percentage of students who “like and enjoy coursework with” mathematics increased slightly from the pre-module survey (16.7%) and to the post-module survey (25.0%) for the 24 students who responded to both surveys. However, there was no statistically significant difference between the distributions of students’ responses in pre-survey and post-survey (Pearson χ^2=0.792, df = 3, p = 0.851, Figure 2).

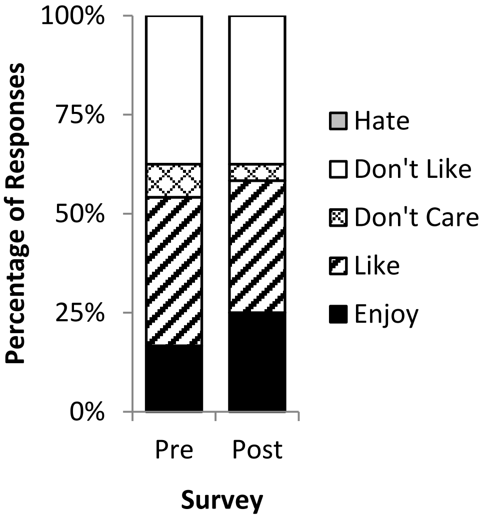


Figure 2. Changes in student responses regarding their attitude towards mathematics (N = 24 students who responded to both pre-module and post-module surveys). No students responded that they "hate and try to avoid" mathematics.

Using only the responses of the 24 students who responded to both pre-module and post-module surveys, which provides the most statistical power, more students responded that mathematics is important to biology, ecology, and conservation biology in post-module surveys than in pre-module surveys (Figure 3). No students responded that mathematics is "not relevant" to biology, ecology, and conservation biology. Only one student responded that mathematics is "not necessary" to biology, ecology, or conservation biology.

This difference was statistically significant for biology (Pearson χ^2=8.096, df = 2, p = 0.017) and ecology (Pearson χ^2=6.857, df = 2, p = 0.032), but not for conservation biology (Pearson χ^2=4.837, df = 3, p = 0.184). The percentage of students who responded that mathematics is "essential" for all work, cutting-edge or not, was highest for conservation biology (79% in post-survey) and lowest for biology (67% in post-survey) in both the pre- and post-surveys.

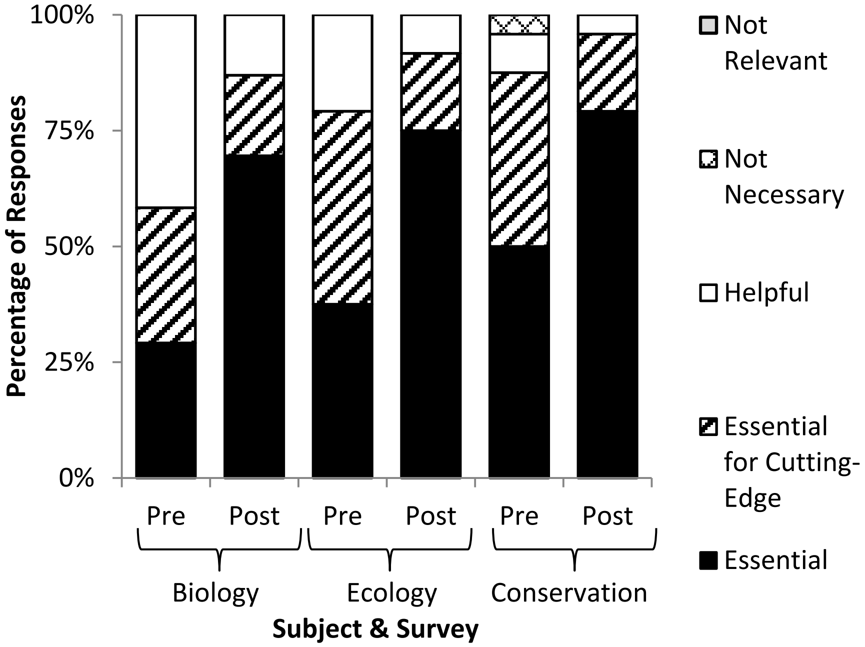


Figure 3. Student responses regarding their attitude towards the importance of mathematics to biology, ecology, and conservation biology (n = 24 students who responded to both surveys).

In open-response questions on the post-module survey, many students made comments such as “This module helped me understand why I was required to take mathematics classes as a marine biology major” and “I assumed you would have to be a mathematics wiz or statistical genius to be able to use models and equations in your research, but now I realize how easy it is to do it yourself.” Another student commented that she had been afraid of using Microsoft Excel, equations, and math, but would definitely take a class on quantitative ecology and would then be more willing to conduct an independent research project.

Although the percentage of students who responded that they would take a quantitative ecology course (if it fit their schedule) increased from 32% (of 19 students) on the pre-survey to 47% (of 32 students) on the post-survey (Figure 4), the change was not statistically significant (Pearson $\chi^2 = 1.764$, df = 2, p = 0.414). I did not use paired responses on this question because I did not ask the question on the pre-survey in 2012 and only 9 students responded to the post-survey in 2013, so I would have had only 9 paired responses.

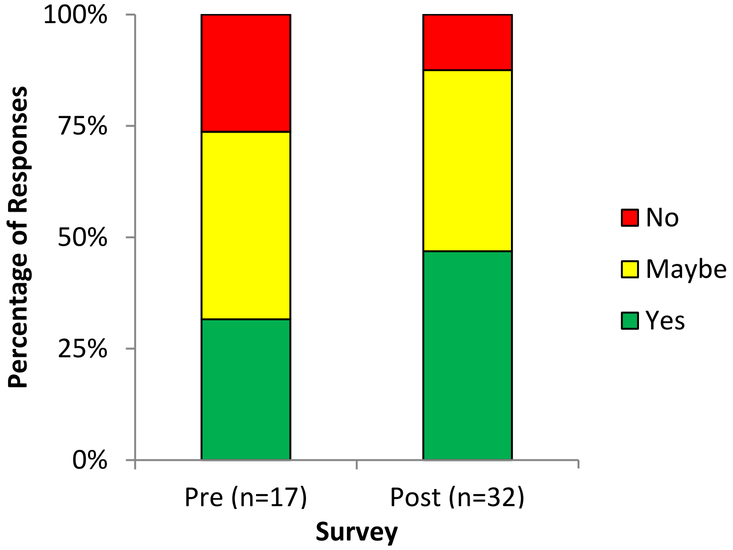


Figure 4. All student responses regarding their willingness to take a quantitative or mathematical ecology course.

A disproportionate number of students who had previously taken the Protected Populations module in BIOE 163 enrolled in the course Quantitative Ecology (BIOE 148) in winter 2010 and 2012. According to Fisher's exact test, this proportion was statistically significantly higher than the proportion of potential students who had taken BIOE 163 in 2010 (67% of 6 students enrolled in BIOE 148, 7% of 393 potential students, odds ratio 29.5, p=0.0003), but not in 2012 (20% of 10 students enrolled in BIOE 148, 11% of 466 potential students, odds ratio 2.02, p = 0.31). Only students enrolled in the three majors contributing to >80% of the enrollment in both classes were included in these calculations.

Learning Key Content: Analysis of Total Scores

Student scores on content questions increased from the pre-survey to the post-survey to the exam. For just those 22 students who responded to all three assessments, a two-factor ANOVA and Tukey's HSD posthoc test showed that there was a significant increase in score between pre-survey and exam ($p = 0.035$), while other differences between year (2012 or 2013), assessment type (pre-survey vs. post-survey, post-survey vs. exam) and the interaction between year and assessment type were not significant (Figure 5).

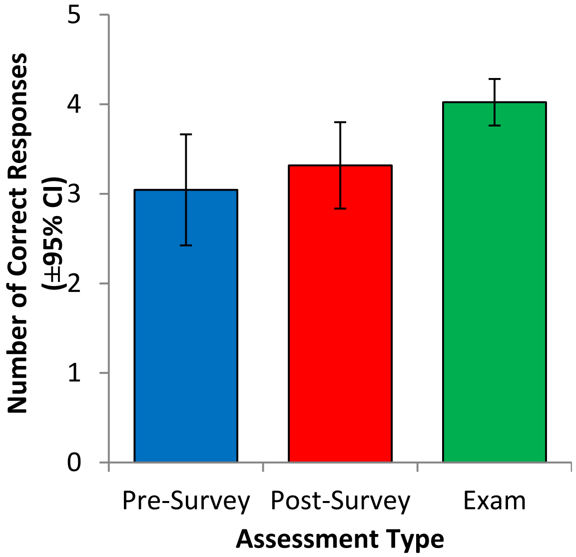


Figure 5. Average number of correct responses ± 95% confidence intervals to five content questions asked in all three assessments, using responses from 22 students who submitted responses to the pre-survey, post-survey, and exam.

When the sample was broadened to include all students, a two-factor ANOVA showed that there were significant differences in assessment type ($p < 0.001$) and in the interaction between assessment type and year ($p = 0.011$). As seen in Figure 6, the interaction reflects the fact that students in 2012 showed much larger gains in learning across the three assessments than did students in 2013, with lower pre-survey scores and higher exam scores in 2012 than in 2013.

The increase in student scores from the pre-survey to the exam resulted in an effect size of 0.83, which is considered a large effect size for an intervention (Cohen 1992).

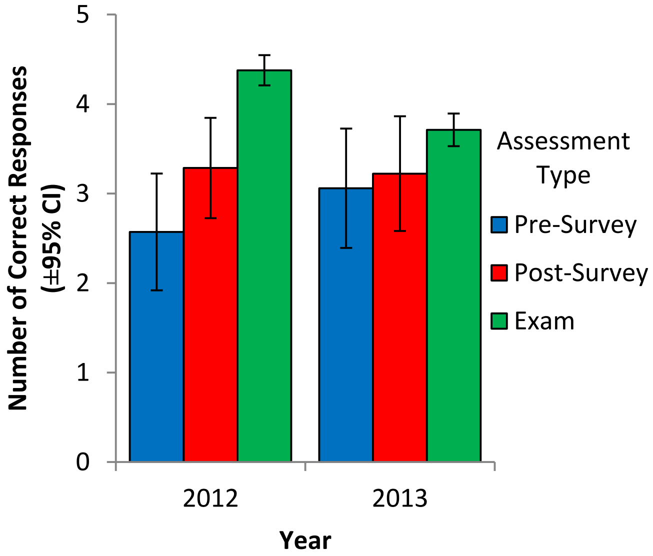


Figure 6. Average number of correct responses ± 95% confidence intervals to five content questions asked in all three assessments, using all responses to pre-survey (21 in 2012, 17 in 2013), post-survey (21 in 2012, 9 in 2013), and exam (61 in 2012, 26 in 2013).

Learning Key Content: Analysis of Questions

The percentage of correct responses increased from the pre-survey to the post-survey to the exam for most questions. 6 of the 7 questions are in sets of 2 questions, in which the first question asked students to use the data to answer a conservation question (interpretation), and the second question asked them to identify the kind of data they used to answer the first question (data choice) (Figure 7). 38 students responded to the pre-survey, 30 students responded to the post-survey, and 87 students responded to the exam.

Question 1 asked students to estimate the population growth rate (λ) with population projection graph provided on surveys and to determine whether the population will increase or decrease with population growth rate provided on the exam. A Pearson chi-square test showed statistically significant differences among all three assessments (Figure 7; $\chi^2=62.342$, $df = 2$, $p < 0.001$), with the highest number of correct responses, as expected, for the exam and the fewest number of correct responses for the post-survey.

The surprisingly low number of correct answers for the post-survey are likely due to differences in the data for this question: the post-survey provided a population projection graph of a population initially growing quickly and eventually reaching an asymptote, in contrast to the data provided for the pre-survey which showed the population continuously increasing and the exam question which used a population growth rate of 1.134. The example used in the post-survey also required a fairly sophisticated understanding of the concept of “transient dynamics”: specifically, a population whose initial stage distribution is substantially different than the stable stage distribution (the asymptotic age distribution associated with a set of vital rates) grows initially but then eventually stabilizes to a lambda of 1 (zero growth) once the stable stage distribution is reached. Thus, the greatest number of students in 2012 (36% of 22 responses) answered that the population growth rate must be “Greater than 1” because the population grew initially rather than “Equal to 1” (18%), which was the best answer, because the population reached an asymptote. The rest of the responses were spread among the other possible responses: “Between 0 and 1”, “Less than 0”, “Less than 1”, “Equal to 0”, and “I don’t know”.

The 2012 interviews indicated confusion as to whether this question meant initial or long-term population growth rate, which may be due to the students’ interpretation of the definition of λ as the ratio between the population size at year t and the population size at year t-1 (a definition used in most ecology textbooks) as opposed to the more explicit algebraic definition of lambda as the asymptotic growth rate associated with a specific demographic schedule and associated set of vital rates. These two definitions of population growth rate (λ) and the resulting confusion highlights the importance of clearly defining all terminology in quantitative biology and the importance of carefully choosing example data in the classroom and on assessments.

In 2013 and in the published module, I emphasized that the population growth rate reflects population growth “after many years with these vital rates,” added the word “long-term” to the post-survey question, and changed the correct response to “Close to 1.” In 2013, 4 of 9 (44%) of post-survey responses were correct, so these changes may have made the point clearer, but students were still more confused about populations that leveled off than they were about populations that continually grew. To further emphasize this point, the published module includes a graph in which the initial and long-term population growth rate differ because the initial population is not in its stable stage distribution.

Question 2 asked students which data they had used to answer Question 1 (population projection graph for pre-survey and post-survey, population growth rate for exam). Although a few students answered incorrectly (they usually answered Question 1 incorrectly as well), most responses to this question had the expected pattern of increases from pre-survey to post-survey to exam. The only statistically significant difference was between the two surveys and the exam (Figure 7; Pearson $\chi^2 = 25.7983$, df = 2, $p < 0.001$), suggesting that studying for the exam helped students answer this question correctly.

Question 3 asked students which life stage could be harvested sustainably (pre-survey), which stage conservation efforts should focus on (post-survey), and which stage managers should try to remove for an invasive species (exam). Students should have used the reproductive value graph to answer that sustainable harvest should focus on stages with the lowest reproductive value to minimize impact on the population; conservation efforts should protect life stages with the highest reproductive value; and managers should try to eliminate life stages of invasive species with the highest reproductive value. Again, the percentage of correct answers increased from pre-survey to post-survey and exam, with a statistically significant difference between the pre-survey and the two post-module assessments (Figure 7; Pearson $\chi^2 = 47.943$, df = 2, $p < 0.001$), suggesting that the module itself was most important, rather than studying for the exam.

Question 4 asked students which data they used to answer Question 3. There are no significant differences in the combined 2012 and 2013 (Figure 7; Pearson $\chi^2 = 2.030$, df = 2, $p = 0.362$), although the percentage of correct responses was highest for the post-survey. This trend was driven mostly by the 2013 data, in which no students answered this question correctly on the exam, even though every single student answered the related interpretation question (Question 3) correctly. In 2012, the percentage of correct responses increased from 50% (of 18 responses) on the pre-survey to 68.2% (of 22 responses) on the post-survey to 74.1% (of 60 responses) on the exam, a similar trend to the other questions. In 2013, half of the students used the elasticity graph and half of the students used the transition matrix to answer the previous interpretation question correctly.

In student interviews, students often stated the misconception that life history stages with the highest fecundity will by definition contribute most to population growth, even though this is not necessarily true according to life history theory (Gotelli 2001). The lecture explicitly covered the concept of reproductive value (a value which is discounted by likelihood of surviving to reproduce) as a measure of the relative contribution of each stage to future population growth, and thus the appropriate statistic with which to answer to the question “Which stage(s) should managers focus monitoring and conservation effort on?” Thus, some students answered the interpretation question by looking at fecundity in the transition matrix (potentially leading to the incorrect answer). Some students also used the elasticity graph because they knew that this graph shows which vital rate conservation efforts should focus on. Students also often used knowledge of the organism’s natural history; for example, on the exam, students usually knew intuitively that removing adult lionfish would have a larger impact than removing juveniles or larvae because relatively few fish survive to become adults. Students were able to correctly answer the interpretation question whether they had used the transition matrix, elasticity graph, or natural history knowledge, because one stage had the highest reproductive value, vital rate with highest elasticity, and highest fecundity in all models used in the assessments. Although this coincidence in one stage is fairly common among organisms due to the calculation of elasticity and reproductive value, instructors may be able to overcome this misconception by discussing an example in which the stage with highest fecundity does not have the highest reproductive value, as in Crouse et al. (1987).

Question 5, which was not included in the exam, asked students which vital rate was most important to protect. The correct answer to this question is the vital rate with highest elasticity. The percentage of correct answers increased from 24% (of 38 responses) on the pre-survey to 53% (of 30 responses) on the post-survey. This was a statistically significant change (Figure 7; Pearson χ^2=6.339, df = 1, p = 0.012).

Question 6, which was not included in the exam, asked students which data they used to answer the Question 5 about vital rates (the correct answer was elasticity graph). The percentage of correct responses increased from 39% (of 38 responses) on the pre-survey to 83% of responses (of 30 responses) on the post-survey (Figure 7), which is a statistically significant change (Pearson χ^2=13.315, df = 1, p < 0.001). Interviews revealed that students often had difficulty interpreting an elasticity matrix, as presented in the activity and interview. Since the elasticity matrix is analogous to and interpreted in the same way as the transition matrix, this likely reflects difficulty interpreting the transition matrix and discomfort with matrices/tables in general. Interviews also revealed that students were confused by the concept of elasticity because they first equated the term with its everyday usage (e.g., rubber bands) and they didn't see how the two definitions were related. As well, several students had learned that elasticity was "the rate of recovery following a disturbance" in a previous course discussing ecosystem resilience (as in Westman 1978), so they were confused by multiple definitions of the same term within ecology courses.

Question 7 required students to interpret the transition matrix by asking (in the surveys) which stage(s) reproduce or (in the exam) in which stage(s) an individual can remain for more than one year. The percentage of correct responses increased from 62% (of 40 responses) on the pre-survey to 70% of (of 32 responses) on the post-survey to 74% (of 86 responses) on the exam (Figure 7). However, this was not a statistically significant change (Pearson χ^2=1.874, df = 2, p = 0.392). 2012 interviews revealed that students confused the survival of an individual with its likelihood of surviving and remaining in the same stage, which some papers and the 2012 lecture called survival and used the symbol S. Thus, in 2013 and the published module, I instead used the term persistence and the symbol P. Students still had trouble interpreting transition matrices, despite extensive discussion of how to convert a transition matrix to a life cycle diagram and vice versa and practice with two simple models.

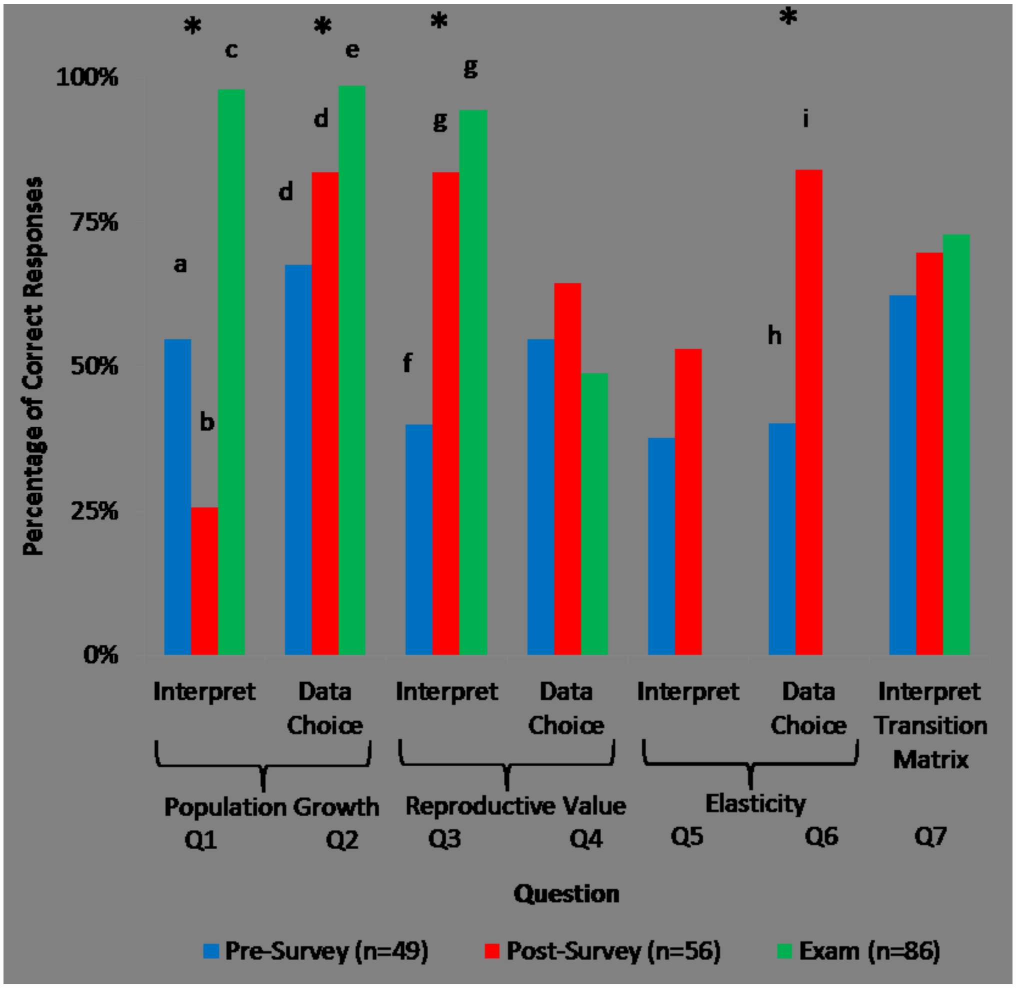


Figure 7. Percentage of correct responses to multiple-choice question asking students to interpret data to answer conservation questions and which form of data they used. Asterisks indicate that Pearson chi-square test determined that there were statistically significant differences ($p < 0.05$). The percentage of correct responses is statistically significantly different between assessments on a particular question if the assessments do not share a letter, according to stepwise Chi-Square Test.

Student Perceptions of Module

Several students commented that the inquiry lab was their favorite part of the module, particularly choosing their organism, asking and answering their own question, calculating the mathematics concepts, and working in small groups. Student comments included:

- "I think the most helpful part of the module was the lab, because we really worked on what the terms, graphs and numbers mean - I think this was more effective in a smaller group."
- "My favorite part was probably having to come up with our own research question, because it made me think about changing the data, and being creative with it to form a hypothesis, and then prove it using the Excel spreadsheet :)!"
- "I liked the part where we made up our own questions and used PopTools to answer it. Honestly at first I thought that was going to be my least favorite and that it was just busy work, but I ended up learning the most from that because I wasn't just doing the work and filling in the blanks of your questions like one usually does with math-related problems."
- "Running the data for ourselves to examine questions we developed was a great exercise, not many classes actually have you apply the mathematics concepts you learn."
- "I liked the fact that we got to choose something we were actually interested in to learn the modeling process with."

Discussion:

After participating in the "Protecting Populations" module, students gained a more positive appreciation of the importance of mathematics in biology and ecology and demonstrated that they learned to apply key uses of structured population models to conservation problems. After the module, students responded that they thought mathematics is far more important to biology, ecology, and conservation biology than they had thought previously. The difference was not statistically significant for conservation biology, perhaps because many students already recognized the importance of mathematics to conservation biology before the module. The percentage of students who responded that they would take a quantitative ecology course increased, although this change was not statistically significant. Students also demonstrated that they learned to apply the concepts of structured population models to specific conservation questions, with a statistically significant difference and large effect size between the number of correct answers to multiple-choice questions on a pre-survey and an exam.

Although this study involved students from only one research university, their experiences in their undergraduate biology curriculum confirm many of the observations in biology education research literature. Most of the students interviewed recalled very few or no biology examples in their mathematics courses, even in courses intended specifically for biology majors. Several students remarked that, by the time they reached upper-division biology courses, they had forgotten everything they learned in the mathematics courses in their first year of college. Others commented that the only mathematics they remember seeing in biology courses was basic exponential and logarithmic growth population models, interpreting graphs, and occasionally a quick introduction to structured population models (but with no lab to help understand it). A few students commented that they skipped any mention of mathematics when reading scientific literature and had to trust the authors' interpretation of their equations, graphs, and tables. Thus, they judged that mathematics was very badly integrated into their undergraduate biology curriculum; it is not surprising that, with this background, nearly 75% of students agreed before taking the module that mathematics is helpful or only essential to cutting edge biology, rather than essential to all biology.

Responding to open-ended post-survey and interview questions, several students commented that the module helped them understand for the first time why their biology majors had required mathematics courses, since their mathematics courses had rarely used biology examples and their biology courses rarely used mathematics. They also commented that the module made them feel much more confident in their own abilities to do the mathematics required of biologists.

Based on responses to survey questions, it appears that the module did not significantly improve most students' perception of mathematics itself or increase their willingness to enroll in a quantitative ecology course. Although enrollment in a quantitative ecology course was extremely low, students completing this module enrolled disproportionately in the course, although this difference was statistically significant in only one of the two quarters it was offered. Matthews et al. (2010) found that an introductory course integrating mathematics and science resulted in students' greater appreciation of the importance of mathematics in biology, but only slightly influenced students' motivation to enroll in quantitative courses. As they remark, the difficulty in persuading students to learn more about quantitative biology suggests that instructors must better integrate more quantitative topics into biology courses and biology departments may need to require quantitative biology courses whose content is essential for life science majors.

Student scores on interpretation of structured population models increased from the pre-survey to the post-survey and exam, with a statistically significant difference and large effect size between the pre-survey and exam, which suggests that both the module itself and students' review of it while studying for the exam were important. Students completing the module in 2012 showed much larger gains in learning across the three assessments than did students completing the module in 2013, despite the larger class size in 2012, possibly because 2013 students were more familiar with the concepts before the module, as evidenced by higher scores on the pre-survey and/or because the 2013 interactive lecture was very rushed due to a shorter time period.

Analysis of student responses to specific questions, in conjunction with interviews, revealed persistent misconceptions about structured populations and specific difficulties in interpretation of structured population models. For most questions, the percentage of correct answers increased from the pre-survey to the post-survey to the exam, although the statistically significant difference sometimes was between pre- and post-survey and sometimes was between pre-survey and exam. Even after considerable emphasis on the long-term meaning of population growth rate in the lecture and in the post-survey question itself, many students retained the alternative conception that the population growth rate must always be greater than 1 if the population initially grows, even if it levels off eventually. It appears that most students had difficulty understanding the relatively sophisticated concept that initial population growth reflects the initial stage distribution, but not necessarily the long-term population growth rate. Most students were able to determine which life stage is most important for conservation effort (question 3), but they often referred to inappropriate data, such as the elasticity graph or transition matrix, rather the correct reproductive value graph. This seems to reflect the alternative conception that the life stage with the highest fecundity must contribute most to population growth, because students had difficulty grasping that high individual fecundity contributes little to population growth if few individuals survive to that stage. Students also had difficulty interpreting the transition matrix to decide which stages had individuals that could reproduce or persist. In order to address these difficulties, instructors may want to emphasize the added graph in the published lecture in which the annual population growth rate varies, discuss an example population in which reproductive value and fecundity are highest in different stages due to low survival (e.g., some fish populations), and provide more practice examples for interpreting transition matrices.

Student reactions to the difficulty level of the module ranged from “It would still take a lot of practice before I could feel completely comfortable working with/interpreting these terms” to “Make [the module] much more advanced because currently it feels like it should be given to middle or elementary students.” However, the range of scores on the exam (Figures 5 and 6) suggests that the difficulty level was appropriate for these students.

My conclusions and student comments are consistent with previous research indicating that learner-centered strategies, such as “Think-Pair-Share”, group brainstorming, case studies, and inquiry labs used in this module, facilitate active learning and increase student interest and motivation, allowing them to gain a deeper understanding of biology as a process of inquiry and learning of biological concepts (e.g., Michael 2006, Knight and Wood 2005, Derting and Ebert-May 2010). Students commented that they particularly liked the inquiry components of the lab, such as choosing their organism, asking and answering their own question, calculating the mathematics concepts, and working in small groups. These learner-centered strategies may particularly help disadvantaged students because Haak et al. (2011) showed that active learning exercises, such as “clicker questions” with peer instruction and extensive informal group work, reduced the achievement gap between disadvantaged and nondisadvantaged students.

These strategies are also likely to help the approximately one-third of college students who have trouble with quantitative work because of “mathematics anxiety” (Betz, 1978). For example, Tobias (1995) recommends helping students with mathematics anxiety by having them work in groups and use computers, calculators, or a list of formulas. Putting mathematics into biology and conservation situations, allowing students to work at a flexible pace, and having the instructor circulating among groups as an approachable facilitator and tutor helps students feel more comfortable with mathematics. As mathematics anxiety is more common among women (Betz 1978), these strategies may particularly help women feel more comfortable with mathematics and using mathematics in biology. Many students, particularly women, in the Protecting Populations module realized, as one student said, that “mathematics is not as hard or intimidating as my mind built it up to be.”

Participating in only one short but carefully-designed and learner-centered module increased students’ recognition of the importance of mathematics in biology, and (not statistically significantly) increased their willingness to take quantitative biology courses, as well as increased their understanding of the key concepts of the module. Instructors of courses throughout the undergraduate biology curriculum should create, utilize, assess, and share modules, like this one, to address important and challenging concepts that illustrate the importance of mathematics to biology and use learner-centered pedagogical strategies. These modules should thoroughly define all terminology in both biological and mathematical terms and address the mathematical modeling process, including making appropriate assumptions and developing a conceptual model prior to adding mathematics (Hodgson et al. 2005, Gobert and Buckley 2000). Developers should also carefully identify and address widespread misconceptions by having students explore and analyze evidence that builds on or counters existing ideas (Tanner and Allen 2005, Smith et al. 1993).

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Appendix:

1. Pre-Module Survey (administered via Google Docs online)
2. Post-Module Survey (administered via Google Docs online, only differences from pre-module survey shown)
3. Exam Questions (administered in lab exam with rotating stations)

Ecological Models Pre-Module Survey

Thank you for taking this survey! This survey is part of a lesson on Ecological Models that includes a lecture on 2/7 and lab on 2/12, which will serve as education research in the dissertation of EEB Ph.D. Candidate Kristin McCully.

Please note that only the researcher (Kristin McCully) will have access to your name in order to match information with your classwork - information will only be presented to others as anonymous quotes or aggregate statistics. The only parts of this module that will count toward your BIOE 163 grade are (1) the worksheet, poster, and presentation in lab 2/12 and (2) questions about ecological models on lab final exam, although you are expected to complete the pre-survey and post-survey.

This survey contains 3 pages with 23 questions (mostly multiple-choice), which should take less than 15 minutes.

Upon completion of this survey, you will be entered into a drawing for a $10 gift certificate to the UCSC Bay Tree Bookstore, which will be announced at lecture on 2/7. Thanks again!

:) Kristin
Top of Form

I. Survey of Basic Information

1. Name:

2. Major:

3. What class level are you in at UCSC?

○ Freshman

○ Sophomore
○ Junior
○ Senior
○ 5th-year
○ Other: ☐

4. Do you plan to pursue a graduate degree in biology?

○ Master’s (M.S.)
○ Ph.D. (possibly preceded by master’s degree)
○ No
○ I don't know
○ Other: ☐

5. What career do you plan to pursue, to the best of your knowledge at this point?

○ Research biologist (not including college/university professor)
○ College/university professor
○ K-12 teacher
○ I don’t know
○ Other: ☐

II. Survey of Attitude Toward Math

1. What is your most recent math class?

○ Math 11A (Calculus with Applications)
○ Math 11B (Calculus with Applications)
○ Math 19A (Calculus for Science, Engineering, and Mathematics)
○ Math 19B (Calculus for Science, Engineering, and Mathematics)
○ Calculus AB AP (Advanced Placement)
○ Calculus BC AP (Advanced Placement)
○ AMS 7 (Biostatistics)
○ Other: ☐

2. How long ago was your most recent math class?

○ < 1 year
○ 1 years
○ 2 years
○ 3 years
○ 4 years
○ Other:

3. Select your grade in your most recent math course:

○ A
○ B
○ C
○ D
○ F
○ Other:

4. Please further describe your math skills and comfort level.

5. Select the statement that most closely represents your attitude toward math:

○ I hate math and try to avoid it.
○ I don’t like math, but I can cope with it.
○ I don’t care about math one way or another.
○ I like math, but I don’t seek it out.
○ I like math and enjoy having coursework that includes math.

6. Select the statement that best describes your attitude regarding the relationship between math and biology:

○ Math is not relevant to biology.

○ Math can be useful in biology, but it’s not really necessary.

○ Math is helpful in biology.

○ Math is essential in biology if you want to do cutting-edge work.

○ Math is essential for doing any biology, cutting-edge or not.

7. Select the statement that best describes your attitude regarding the relationship between math and ecology:

○ Math is not relevant to ecology.

○ Math can be useful in ecology, but it’s not really necessary.

○ Math is helpful in ecology.

○ Math is essential in ecology if you want to do cutting-edge work.

○ Math is essential for doing any ecology, cutting-edge or not.

8. Select the statement that best describes your attitude regarding the relationship between math and conservation biology:

○ Math is not relevant to conservation biology.

○ Math can be useful in conservation biology, but it’s not really necessary.

○ Math is helpful in conservation biology.

○ Math is essential in conservation biology if you want to do cutting-edge work.

○ Math is essential for doing any conservation biology, cutting-edge or not.

9. Would you take a course on quantitative or mathematical ecology?

○ Yes, if it fit my schedule

○ Maybe

○ No

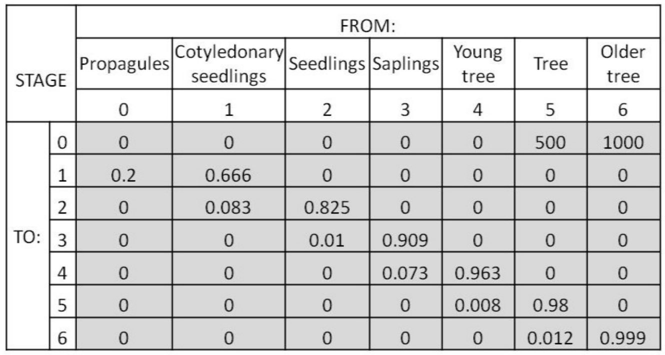
○ Other: ☐

10. Please expand on whether you feel math is important to biology, ecology, and/or conservation biology.

III. Survey of Ecological Models Module Skills

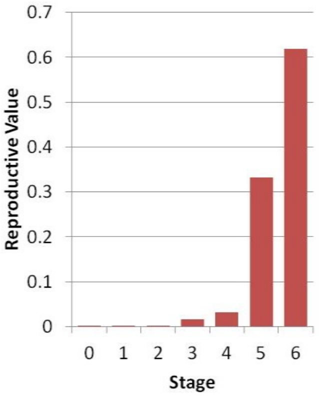
The purpose of this section of the survey is to determine how much you already know before the module. You are not expected to know any of the terms or information below. It will be covered in the module. If you do not know or at least have a strong guess, please answer "I don't know!" The accompanying data is for a population of *Avicennia marina* mangroves in southeastern Australia. To access the data needed to answer the following questions, open the link below by copying the link into a new window: http://bio.classes.ucsc.edu/bioe163/data.html

Transition Matrix

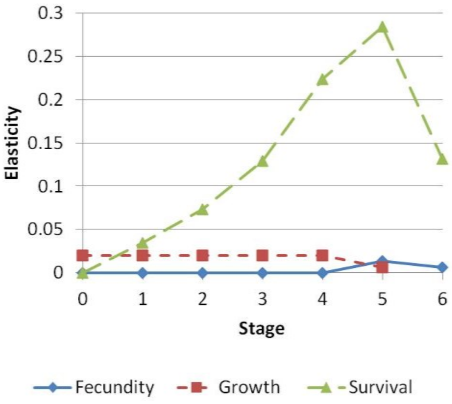


STAGE		FROM:						
		Propagules	Cotyledonary seedlings	Seedlings	Saplings	Young tree	Tree	Older tree
		0	1	2	3	4	5	6
TO:	0	0	0	0	0	0	500	1000
	1	0.2	0.666	0	0	0	0	0
	2	0	0.083	0.825	0	0	0	0
	3	0	0	0.01	0.909	0	0	0
	4	0	0	0	0.073	0.963	0	0
	5	0	0	0	0	0.008	0.98	0
	6	0	0	0	0	0	0.012	0.999

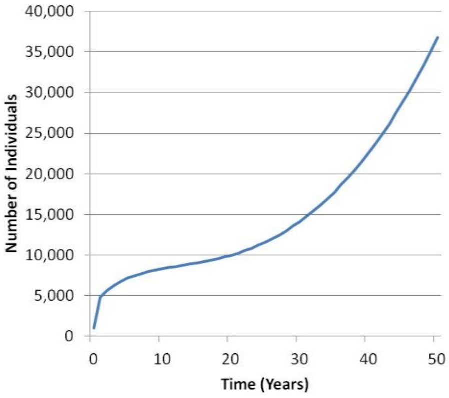
Reproductive Value Graph



Elasticity Graph



Population Projection Graph



According to the data provided, the population growth rate (λ) must be:

○ Less than 0
○ Equal to 0
○ Between 0 and 1
○ Equal to 1
○ Greater than 1
○ I don't know!

Which form of data is most useful for answering question 1?

○ Transition matrix
○ Reproductive value graph
○ Elasticity graph
○ Population projection graph
○ I don't know!
○ Other: ________

A nearby town wants to start harvesting mangrove trees, but wants to make sure that their harvest is sustainable. You use the model to recommend that they harvest:

○ Stage 4 (Young tree)
○ Stage 5 (Tree)
○ Stage 6 (Older tree)
○ I don't know!
○ Other: ________

Which form of data is most useful for answering question 3?

○ Transition matrix
○ Reproductive value graph
○ Elasticity graph
○ Population projection graph
○ I don't know!
○ Other: ________

Which vital rate is most important to protect?

○ Fecundity of Stage 6

○ Survival of Stage 6

○ Survival of Stage 5

○ Growth of Stage 5

○ I don't know!

○ Other: ☐

Which form of data is most useful for answering question 5?

○ Transition matrix

○ Reproductive value graph

○ Elasticity graph

○ Population projection graph

○ I don't know!

○ Other: ☐

According to the life history transition matrix, which stages reproduce?

○ Stages 3, 4, 5, and 6

○ Stages 4, 5, and 6

○ Stages 5 and 6

○ Only stage 6

○ I don't know!

○ Other: ☐

If you are familiar with these terms, please describe how you learned them.Population growth rate, transition matrix, reproductive value, elasticity

Ecological Models Post-Module Survey

I. Survey of Basic Information

(Questions 1-5 are identical to pre-module survey)
6. Did you attend the lecture on Ecological Models on 2/9/13?

○ Yes

○ No

II. Survey of Attitude Toward Math

(Questions 1-9 are identical to pre-module survey except that the word "NOW" was added to end of questions 5-9)

10. Please describe how and if the Ecological Models module changed how you view the importance of math to biology, ecology, or conservation biology.

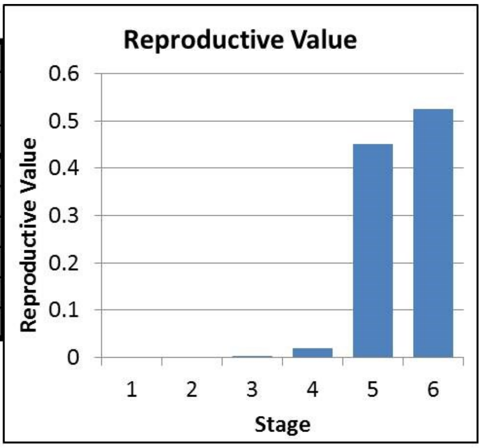
11. Which part(s) of the module did you like best? Like least?

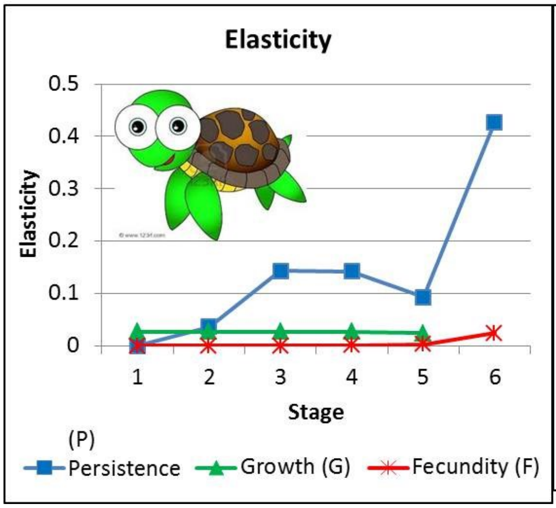
12. Do you have any suggestions to improve the Ecological Models module?

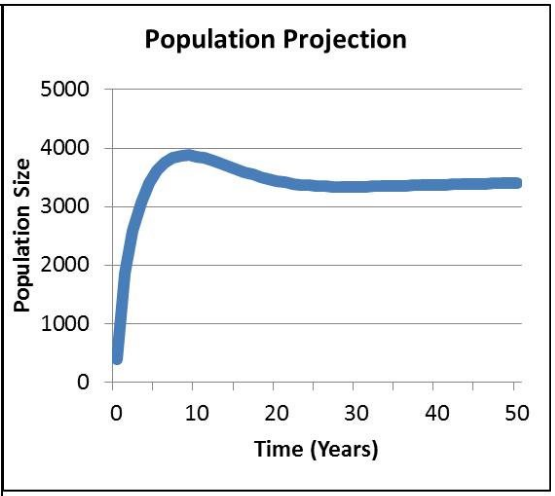
III. Survey of Ecological Models Module Skills

The accompanying data is for a population of green sea turtles (*Chelonia mydas*) of the southern Great Barrier Reef, Australia (Chaloupka 2001 Ecological Modelling). To access the data needed to answer the following questions, open the link below by copying the link into a new window: http://bio.classes.ucsc.edu/bioe163/data_post.html

		FROM:					
		Egg/ Hatchling	Pelagic Juvenile	Benthic Juvenile	Subadult	Maturing Adult	Adult
		1	2	3	4	5	6
TO:	1	0	0	0	0.2488	40.5916	100
	2	0.4394	0.5704	0	0	0	0
	3	0	0.0741	0.8413	0	0	0
	4	0	0	0.0291	0.8405	0	0
	5	0	0	0	0.0069	0.7782	0
	6	0	0	0	0	0.17	0.9482







1. According to the data provided, the long-term population growth rate (λ) must be:

○ Less than 0

○ Equal to 0

○ Between 0 and 1

○ Close to 1

○ Greater than 1

○ I don't know!

2. Which form of data is most useful for answering question 1?

○ Transition matrix
○ Reproductive value graph
○ Elasticity graph
○ Population projection graph
○ I don't know!
○ Other:

3. A conservation non-profit organization has one million dollars to protect this turtle population. You recommend that they spend their money to protect:

○ Stage 1: Egg/Hatchling
○ Stages 3 & 4: Benthic Juvenile & Subadult
○ Stages 5 & 6: Maturing Adult & Adult
○ I don't know!
○ Other:

4. Which form of data is most useful for answering question 3?

○ Transition matrix
○ Reproductive value graph
○ Elasticity graph
○ Population projection graph
○ I don't know!
○ Other:

5. Which vital rate is most important to protect?

○ Fecundity of Stage 6
○ Survival of Stage 6
○ Survival of Stage 5
○ Growth of Stage 5
○ I don't know!
○ Other:

6. Which form of data is most useful for answering question 5?

○ Transition matrix
○ Reproductive value graph
○ Elasticity graph
○ Population projection graph
○ I don't know!
○ Other: ☐

7. According to the life history transition matrix, which stages reproduce (at all)?

○ Stages 3, 4, 5, and 6
○ Stages 4, 5, and 6
○ Stages 5 and 6
○ Only stage 6
○ I don't know!
○ Other: ☐

8. If you were familiar with these terms before this module, please describe how you learned them. Feel free to skip if you answered this question in pre-survey. Population growth rate, transition matrix, reproductive value, elasticity

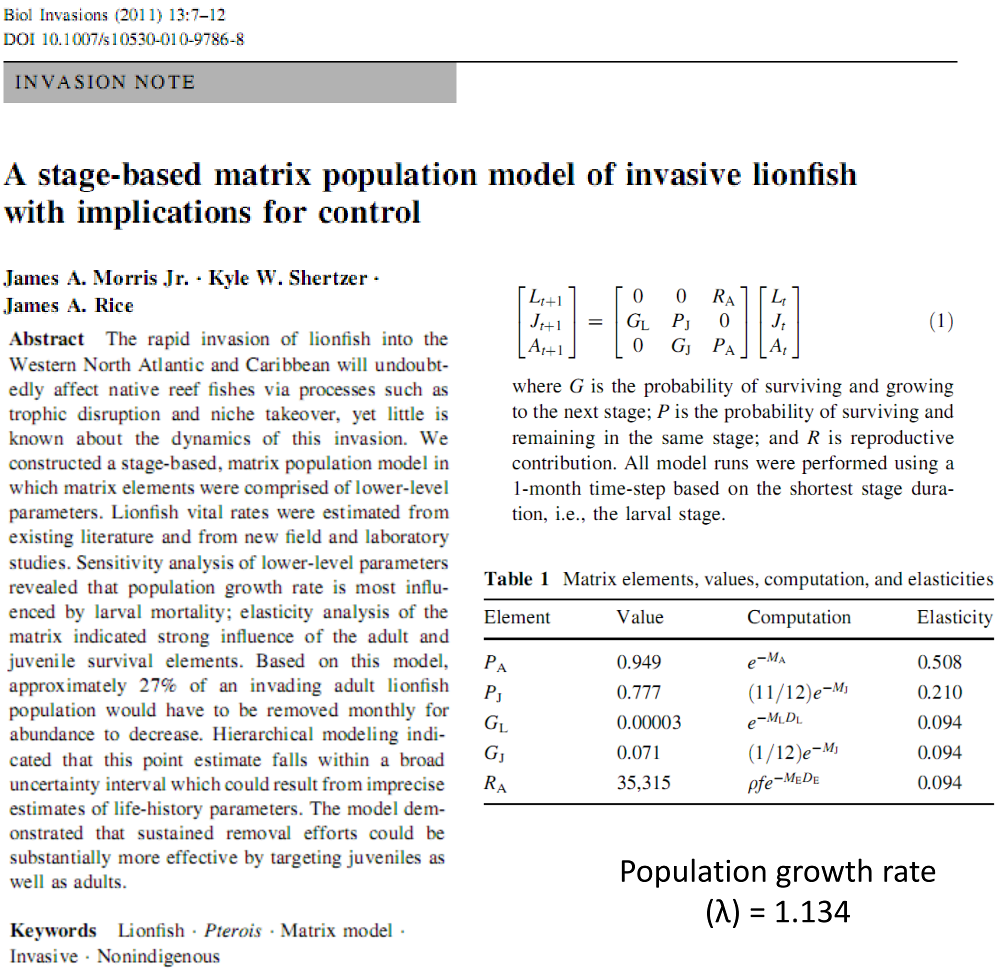
9. Do you feel more comfortable with these terms and interpreting their numbers and graphs after the module? Please describe what has changed and which part(s) of the module helped most.

Exam Questions

Stations 1 & 2: Ecological Models

Invasive lionfish (*Pterois miles* and *P. volitans*) are now established along the southeast coast of the United States and parts of the Caribbean. Lionfish represent the first marine reef fish invader to this region and are thought to have been released intentionally by aquarists over many years. The rapidity of the lionfish invasion in the Western North Atlantic and Caribbean is unprecedented among marine fishes. Not only are invasive lionfish now widely distributed geographically, in some locations they are one of the most abundant reef fishes.

Use the datasheet provided from Morris et al. (2011) to answer the following questions.



Biol Invasions (2011) 13:7–12
DOI 10.1007/s10530-010-9786-8

INVASION NOTE

A stage-based matrix population model of invasive lionfish with implications for control

James A. Morris Jr. · Kyle W. Shertzer · James A. Rice

Abstract The rapid invasion of lionfish into the Western North Atlantic and Caribbean will undoubtedly affect native reef fishes via processes such as trophic disruption and niche takeover, yet little is known about the dynamics of this invasion. We constructed a stage-based, matrix population model in which matrix elements were comprised of lower-level parameters. Lionfish vital rates were estimated from existing literature and from new field and laboratory studies. Sensitivity analysis of lower-level parameters revealed that population growth rate is most influenced by larval mortality; elasticity analysis of the matrix indicated strong influence of the adult and juvenile survival elements. Based on this model, approximately 27% of an invading adult lionfish population would have to be removed monthly for abundance to decrease. Hierarchical modeling indicated that this point estimate falls within a broad uncertainty interval which could result from imprecise estimates of life-history parameters. The model demonstrated that sustained removal efforts could be substantially more effective by targeting juveniles as well as adults.

Keywords Lionfish · *Pterois* · Matrix model · Invasive · Nonindigenous

$$\begin{bmatrix} L_{t+1} \\ J_{t+1} \\ A_{t+1} \end{bmatrix} = \begin{bmatrix} 0 & 0 & R_A \\ G_L & P_J & 0 \\ 0 & G_J & P_A \end{bmatrix} \begin{bmatrix} L_t \\ J_t \\ A_t \end{bmatrix} \qquad (1)$$

where G is the probability of surviving and growing to the next stage; P is the probability of surviving and remaining in the same stage; and R is reproductive contribution. All model runs were performed using a 1-month time-step based on the shortest stage duration, i.e., the larval stage.

Table 1 Matrix elements, values, computation, and elasticities

Element	Value	Computation	Elasticity
P_A	0.949	e^{-M_A}	0.508
P_J	0.777	$(11/12)e^{-M_J}$	0.210
G_L	0.00003	$e^{-M_L D_L}$	0.094
G_J	0.071	$(1/12)e^{-M_J}$	0.094
R_A	35,315	$\rho f e^{-M_E D_E}$	0.094

Population growth rate
(λ) = 1.134

Stage	Stable Stage Distribution	Reproductive Value
1. Larvae	99.9884%	0.0
2. Juvenile (<174 mm)	0.0084%	0.166
3. Adult	0.0032%	0.834

Lionfish larvae from Cancun, 2010 (NOAA SE Fisheries Science Center)

Lionfish juvenile captured at Long Island (NY) in 2001
(Todd Gardner, http://www.advancedaquarist.com/2006/6/aafeature)

1. If this population growth rate continues for many years, this population will
   a. Rapidly disappear
   b. Slowly disappear
   c. Remain at the same size
   d. Increase

2. Which form of data is most useful for answering question A?

   a. Transition matrix
   b. Elasticity (in Table 1)
   c. Population growth rate
   d. Stable stage distribution
   e. Reproductive value

3. In order to reduce or eliminate this invasive species, managers should attempt to remove
   a. Only larvae
   b. Only adults
   c. Only juveniles
   d. Mostly adults, but also juveniles

4. Which form of data is most useful for answering question C?
   a. Transition matrix
   b. Elasticity (in Table 1)
   c. Population growth rate
   d. Stable stage distribution
   e. Reproductive value

5. Write the numbers of the stage(s) in which a lionfish can remain for only one year (or none).

6. Write the proportion of adults who survive each year.

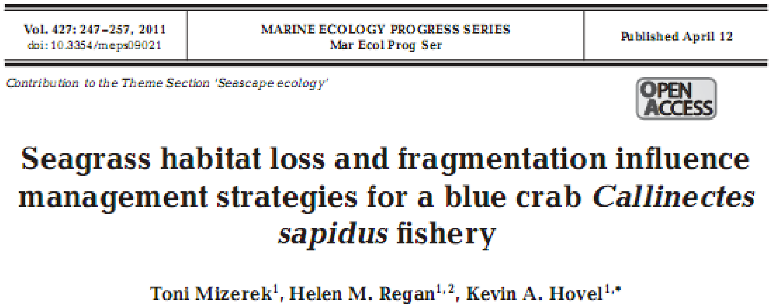
7. Write the proportion of larvae who survive each year.

8. In which stage(s) will most of the population be, after many years?

9. Which form of data is most useful for answering question B?
   a. Transition matrix
   b. Elasticity (in Table 1)
   c. Population growth rate
   d. Stable stage distribution
   e. Reproductive value

Station 3: Ecological Models

Use the datasheet provided from Mizerek et al. (2011) to answer the following questions about the blue crab (*Callinectes sapidus*) population of Chesapeake Bay, which lives in eelgrass beds. This is a more complicated population model with two seasons, but you should be able to use your skills at evaluating and translating transition matrices to understand it.
Note: megalopae = final larval stage of true crabs.



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doi: 10.3354/meps09021

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published April 12

Contribution to the Theme Section 'Seascape ecology'

OPEN ACCESS

Seagrass habitat loss and fragmentation influence management strategies for a blue crab *Callinectes sapidus* fishery

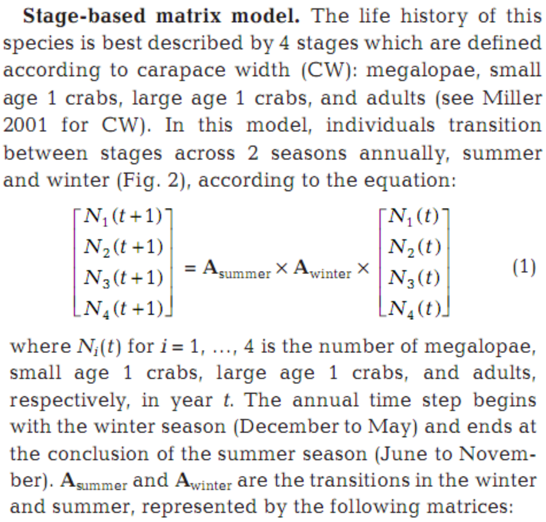
Toni Mizerek[1], Helen M. Regan[1,2], Kevin A. Hovel[1,*]

Blue crabs (Wikipedia)

From Introduction (references removed):

The blue crab (*Callinectes sapidus)* forms one of the most valuable single-species fisheries along the east coast and gulf coasts of the USA; however, in estuaries such as Chesapeake Bay, blue crab populations are in decline at least in part due to excessive fishing pressure. Moreover, eelgrass *Zostera marina*, the primary nursery habitat for blue crab in Chesapeake Bay, has declined by 80% from historical levels due to non-anthropogenic (e.g. storms, digging predators) and anthropogenic (e.g. nutrient loading, sedimentation, propeller scarring) causes.

Our goal in this study was to determine how the loss and fragmentation of seagrass habitat may influence the success of different blue crab management scenarios (reductions in harvest and stock enhancement; blue crabs are hatchery-reared and released into the bay as a stock enhancement strategy).

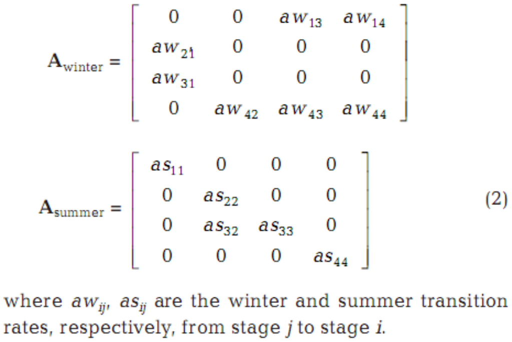


Stage-based matrix model. The life history of this species is best described by 4 stages which are defined according to carapace width (CW): megalopae, small age 1 crabs, large age 1 crabs, and adults (see Miller 2001 for CW). In this model, individuals transition between stages across 2 seasons annually, summer and winter (Fig. 2), according to the equation:

$$\begin{bmatrix} N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \\ N_4(t+1) \end{bmatrix} = \mathbf{A}_{\text{summer}} \times \mathbf{A}_{\text{winter}} \times \begin{bmatrix} N_1(t) \\ N_2(t) \\ N_3(t) \\ N_4(t) \end{bmatrix} \qquad (1)$$

where $N_i(t)$ for $i = 1, \ldots, 4$ is the number of megalopae, small age 1 crabs, large age 1 crabs, and adults, respectively, in year t. The annual time step begins with the winter season (December to May) and ends at the conclusion of the summer season (June to November). $\mathbf{A}_{\text{summer}}$ and $\mathbf{A}_{\text{winter}}$ are the transitions in the winter and summer, represented by the following matrices:

Megalopa of blue crab
http://www.dnr.state.md.us/fisheries/megalops.html



$$\mathbf{A}_{\text{winter}} = \begin{bmatrix} 0 & 0 & aw_{13} & aw_{14} \\ aw_{21} & 0 & 0 & 0 \\ aw_{31} & 0 & 0 & 0 \\ 0 & aw_{42} & aw_{43} & aw_{44} \end{bmatrix}$$

$$\mathbf{A}_{\text{summer}} = \begin{bmatrix} as_{11} & 0 & 0 & 0 \\ 0 & as_{22} & 0 & 0 \\ 0 & as_{32} & as_{33} & 0 \\ 0 & 0 & 0 & as_{44} \end{bmatrix} \qquad (2)$$

where aw_{ij}, as_{ij} are the winter and summer transition rates, respectively, from stage j to stage i.

1. Use the transition matrices to write which stages of blue crabs reproduce.

2. Use the transition matrices to write which season blue crabs reproduce (summer or winter).

3. Mizerek et al. (2011) determined that small age 1 survival in winter is much lower in eelgrass patches that are large and isolated (~0.15) than eelgrass patches that are small and close together (~0.42) possibly due to greater predator abundance (graph below). Write the symbol(s) from the matrix that refer to this vital rate.

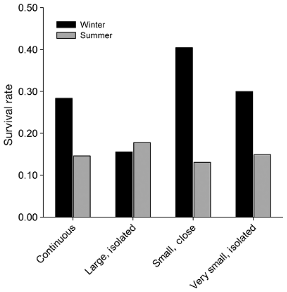


Fig. 3. *Callinectes sapidus*. Winter and summer small age 1 blue crab survival rates per habitat fragmentation type (Hovel & Lipcius 2002, K. A. Hovel unpubl. data)

4. Use the symbols in the transition matrices to write an expression (e.g., 2x + y – 3z) for mortality of small age 1 crabs in summer.