

**Population dynamics of clownfish sea anemones:  
Patterns of decline, symbiosis with anemonefish, and management for sustainability**

by

Matthew James McVay

A thesis submitted to the Graduate Faculty of  
Auburn University  
in partial fulfillment of the  
requirements for the Degree of  
Master of Science

Auburn, Alabama  
December 12, 2015

Keywords: sea anemone, population dynamics, *Entacmaea quadricolor*, *Heteractis  
crispa*, sustainable management, Red Sea coral reefs

Copyright 2015 by Matthew James McVay

Approved by

Nanette E. Chadwick, chair, Associate Professor of Biological Sciences  
F. Stephen Dobson, Professor of Biological Sciences  
Todd D. Steury, Associate Professor of Forestry and Wildlife Sciences

## **Abstract**

Giant sea anemones on Indo-Pacific coral reefs are important ecologically as hosts to obligate clownfish (anemonefish) and cleanershrimp symbionts, and also economically as major components of the global trade in reef invertebrates for ornamental aquaria. The population dynamics of these sea anemones remain poorly understood, but are vital to their sustainable management. I applied size-based demographic models to population information collected intensively during 1996-2000, and then again in 2013-2014, for the 2 major species of clownfish sea anemones on coral reefs at Eilat, Israel, northern Red Sea. High rates of mortality led to highly dynamic populations of both species. In turn, relatively low recruitment caused gradual population decline, which continued through 2014, when the abundances of both the anemone hosts and their fish associates were at all-time lows. The long-term decline of habitable sea anemones observed here significantly altered the anemonefish population structure, creating a negative feedback loop in which the fish changes then impacted their mutualistic hosts. Based on this long-term demographic analysis, including patterns of elasticity and population turnover, I provide recommendations for the sustainable harvest of these giant sea anemones on coral reefs. This demographic study reveals the processes leading to population decline in an important coral reef mutualism, as well as the urgent need for more scientifically-based management to prevent the local extinction of both mutualistic partners on these reefs.

## Acknowledgements

An abundance of gratitude is owed to my advisor, Dr. Nanette Chadwick. She took a chance on me towards the end of my undergraduate degree by providing me an opportunity to study clownfish and sea anemone demography in the Red Sea at Aqaba, Jordan during the summer of 2010. Following that trip, her support was very influential as we continued our work on sea anemone population dynamics after my graduation and into the start of my graduate work in the spring of 2013. I will always be grateful for Dr. Chadwick's insight and direction, and for pushing me to be a better scientist and writer. I also thank my committee members, Dr. Stephen Dobson and Dr. Todd Steury, for their collaboration and guidance in the development of my thesis. My following lab mates were also greatly appreciated for their comradery and collaboration: Erin O'Reilly, Jessica Gilpin, Mark Stuart, Channing Cantrell, Bobbie Renfro, and Alexandra Colombara.

A special thanks goes to my parents, who prepared me for life and have always provided much support and love for me. Any success I have stems from the foundation they built for me. I also have much gratitude for the support of my loving in-laws. Finally, I thank Emily, the love of my life, and James, my son, who have been an enormous source of daily encouragement and excitement for me. I love you two very dearly, and am so thankful that God has blessed me far beyond what I deserve in letting me experience life with you.

## Table of Contents

Abstract.....	ii
Acknowledgments .....	iii
List of Tables .....	v
List of Figures.....	vi
Chapter 1 — Sea anemone population dynamics: Overview of demographic analyses and sustainable management issues.....	1
Chapter 2 — Population dynamics of clownfish sea anemones at Eilat, northern Red Sea: Implications for sustainable management.....	6
Abstract.....	6
Introduction.....	7
Methods.....	12
Results.....	19
Discussion.....	27
References.....	41
Chapter 3 — Long-term decline of clownfish sea anemones in the Israeli Red Sea and effects on obligate anemonefish associates.....	63
Abstract.....	63
Introduction.....	64
Methods.....	66
Results.....	69
Discussion.....	72
References.....	79

## List of Tables

Table 2.1	Annual transition matrices over 4 years for 2 species of sea anemones .....	51
Table 2.2	Supplemental transition matrices with recruitment rates added in .....	52
Table 2.3	Elasticity values for <i>Entacmaea quadricolor</i> over 4 years .....	53
Table 2.4	Elasticity values for <i>Heteractis crispa</i> over 4 years .....	54
Table 2.5	Variation in rates of population turnover time among years, species.....	55
Table 3.1	Decline of 2 sea anemones and their anemonefish from 1996-2014.....	81
Table 3.2	Variation in mean TCSA within size classes, among years .....	82
Table 3.3	Variation in the number of sea anemones that hosted differing levels of anemonefish .....	83
Table 3.4	Variation in the number of anemonefish in the 2 host species .....	84
Table 3.5	Breeding pairs of anemonefish hosted by <i>Entacmaea quadricolor</i> .....	85

## List of Figures

Figure 2.1	Map of the northern Gulf of Aqaba, Red Sea, with study site location .....	56
Figure 2.2	Schematic illustration of the 3 sea anemone size classes, and the parameters that control their size-frequency distributions .....	57
Figure 2.3	Variation in total abundance sea anemones and recruits .....	58
Figure 2.4	Population size structure of sea anemones among years and species .....	59
Figure 2.5	Variation in rates of recruitment and mortality by year and species .....	60
Figure 2.6	Variation in patterns of change in body size in <i>Entacmaea quadricolor</i> ...	61
Figure 2.7	Variation in patterns of change in body size in <i>Heteractis crispa</i> .....	62
Figure 3.1	Population size structure of sea anemones from 2013-2014.....	86
Figure 3.2	Variation in actual versus predicted abundance values over 20 years .....	87

## Chapter 1

### **Sea anemone population dynamics: Overview of demographic analyses and sustainable management issues**

The symbiosis between coral reef sea anemone hosts and their anemonefish (clownfish) associates was first reported nearly 150 years ago (Collingwood 1868). Several ecological benefits attributed to the clownfish-anemone mutualism have since been revealed by scientists (reviewed in Fautin and Allen 1997, Szczebak et al. 2013). In addition to coral reef investigators, the general public is well-acquainted with this fascinating association, which has become a common example of symbiotic relationships among organisms, especially through the popular media as exemplified by the 2003 Pixar film *Finding Nemo*.

This clownfish-anemone symbiosis exhibits ~30 species of obligate anemonefishes who associate with 10 species of giant sea anemone hosts on Indo-Pacific coral reefs (Fautin and Allen 1997). Despite the importance of this association in enhancing biodiversity on coral reefs, little is known about the biology of the host sea anemones. Most of the published information about this symbiosis concerns the fish associates, or the interaction between fish and host, which is most commonly viewed as a defense mutualism. The sea anemone protects its fish associates from predators with an array of tentacles laden with venomous stinging cells called nematocysts. In turn, the often aggressive anemonefish protects its host from predation by attacking organisms like butterflyfish and sea turtles, which attempt to feed on the sea anemone tentacles (Porat and Chadwick-Furman 2004). Other known benefits that anemonefish provide to sea

anemones include supplemental nitrogen and oxygenation, which aid in tissue growth and reproduction (Roopin and Chadwick 2009, Szecebak et al. 2013). Additionally, much is known about the life histories of the anemonefish (Simpson et al. 2014). Almost no information exists concerning the population dynamics of giant clownfish sea anemones, even though they are collected intensively for the ornamental aquarium trade—and sometimes overharvested—in many places around the world. A decade-old study on sea anemone collection in the Philippines reported that clownfish sea anemones were harvested at rates that cannot be matched by their reproduction, and that harvest rates must be adjusted to avoid further population declines and local extinctions (Shuman 2005). Coral reef sea anemones also are potentially affected by environmental stressors such as rising sea water temperature, habitat destruction, sedimentation, pollution, and local nutrient enrichment that contribute to reef degradation (Szmant 2002, Thornhill 2012, Hobbs et al. 2013). Quantitative, long-term demographic studies on clownfish sea anemone populations are needed as a scientific basis to support sustainable management and harvest practices for these vulnerable symbiotic hosts.

In Chapter 2 of this thesis, I describe the population dynamics of 2 clownfish sea anemones in the northern Red Sea, and make the first extensive management recommendations for their sustainable harvest. The analysis reported was based on annual examination of their population structure, growth, shrinkage, mortality, and recruitment during 1996-2000 on coral reefs at Eilat, Israel. Because sea anemones possess no hard skeleton, and not only grow but shrink, standard age-based population models are difficult to apply. Thus, I followed Hughes' (1984) size-based demographic model, modified from a classic age-based model, for the 2 populations of sea anemones

at Eilat. Transition matrices were used to reveal probabilities of vital rates for the individual anemones within each designated size class. Understanding how individuals transitioned within the population provided insights into their whole-population dynamics, as well as how those populations can be expected to respond in similar conditions in the future. Both sea anemone populations declined substantially during 1996-2000, allowing me to compare those short-term trends with their current population abundances and to analyze the more recent, long-term effects of their demographic patterns over almost 20 years. Elasticity analyses were applied to determine the transition probabilities that have the greatest effect on population growth, and population turnover time was calculated as the time required for complete replacement of individuals at a given time.

In Chapter 3, I present recent demographic information for both sea anemone populations collected in follow-up surveys at the same study site during 2013-2014. By comparing the trends observed during 1996-2000 to those in 2013-2014, I tested the population projections that arose from the original dataset, providing insight into the important parameters that shape long-term population trends in these 2 host anemone species. Because the anemonefish associates potentially benefit both hosts by providing predator defense and supplemental nutrients (see above), the fish dynamics also were described, as well as their effects on host body size and survivorship. As the sea anemone populations continued to decline, the anemonefish populations also waned over the long-term study at this site, but with interesting patterns of altered population structure that depended on the host species.

The recent trends and analyses reported in Chapter 3 reinforce and supplement the more intensive, earlier population assessment detailed in Chapter 2. Together, these 2 studies provide the first long-term, in-depth study of demography in clownfish sea anemones, and form a strong scientific basis for the sustainable population management of these important coral reef organisms.

## References

- Collingwood, C. (1868) IV.—Note on the existence of gigantic sea-anemones in the China Sea, containing within them quasi-parasitic fish. *Journal of Natural History*, 1(1):31-33.
- Fautin, D.G., Allen, G.R. (1997) *Anemonefishes and their Host Sea Anemones: A Guide for Aquarists and Divers*. Revised edition. Western Australian Museum, Perth, 160 pp.
- Hobbs, J.P.A., Frisch, A.J., Ford, B.M., Thums, M., Saenz-Agudelo, P., Furby, K.A., Berumen, M.L. (2013) Taxonomic, spatial and temporal patterns of bleaching in anemones inhabited by anemonefishes. *PLoS ONE* 8(8):e70966.
- Hughes, T.P. (1984) Population dynamics based on individual size rather than age: a general model with a reef coral example. *American Naturalist* 123(6):778-795.
- Porat, D., Chadwick-Furman, N.E. (2004) Effects of anemonefish on giant sea anemones: expansion behavior, growth, and survival. *Hydrobiologia* 531:513–520.
- Roopin, M., Chadwick, N.E. (2009) Benefits to host sea anemones from ammonia contributions of resident anemonefish. *Journal of Experimental Marine Biology and Ecology* 370:27-34.
- Simpson, S.D., Harrison, H.B., Claereboudt, M.R., Planes, S. (2014) Long-distance dispersal via ocean currents connects Omani clownfish populations throughout entire species range. *PLoS ONE* 9(9):e107610.
- Shuman, C.S., Hodgson, G., Ambrose, R.F. (2005) Population impacts of collecting sea anemones and anemonefish for the aquarium trade in the Philippines. *Coral Reefs* 24:564-573.
- Szczebak, J.T., Henry, R.P., Al-Horani, F.A., Chadwick, N.E. (2013) Anemonefish oxygenate their anemone hosts at night. *Journal of Experimental Biology* 216:970-976.
- Szmant, A.M. (2002) Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? *Estuaries* 25(4):743-766.
- Thornhill, D.J. (2012) *Ecological Impacts and Practices of the Coral Reef Wildlife Trade*. Defenders of Wildlife, Washington D.C., 179 pp.  
<http://www.defenders.org/sites/default/files/publications/ecological-impacts-and-practices-of-the-coral-reef-wildlife-trade.pdf>.

## Chapter 2

### Population dynamics of clownfish sea anemones at Eilat, northern Red Sea:

#### Implications for sustainable management

#### Abstract

Giant sea anemones on Indo-Pacific coral reefs are important ecologically as hosts to obligate clownfish (anemonefish) and cleanershrimp symbionts, and also economically as major components of the global trade in reef invertebrates for ornamental aquaria. The population dynamics of these coral reef sea anemones remain poorly understood, but are vital to their sustainable management. We determined demographic processes in the 2 main host species for clownfish in the Red Sea, the bulb-tentacle sea anemone *Entacmaea quadricolor* and the leathery sea anemone *Heteractis crispa*, over 4 years at Eilat, Israel. We applied stage-based demographic models and grouped anemones by body size to assess variation in patterns of recruitment, growth, shrinkage and mortality. Populations of both species were highly dynamic, and declined markedly in abundance over 4 years with low recruitment and high mortality occurring in some years. Matrix transitions by both the smallest and largest individuals (depending on year) contributed the most to population change. Population turnover rate was more rapid than expected for these large sea anemones, estimated at complete replacement of individuals in 7 and 5 years for *E. quadricolor* and *H. crispa*, respectively. Based on their population dynamics and ecological interactions, we make the following management recommendations for

the sustainable harvest of these 2 important sea anemone species: (1) seasonal closure of harvest during their estimated annual spawning periods, to allow especially the largest individuals to release abundant gametes, (2) designation of nearby protected areas where anemones are not harvested, preferably up-current from harvested (or otherwise degraded) reefs, to support local recruitment and population replenishment, (3) a slot limit that allows collection of only medium-sized individuals, which have relatively little impact on population changes, and (4) protection from harvest of interspecific pairs, in which nursery anemones *H. crispera* are located < 5 m distance from breeding fish hosts *E. quadricolor*, to allow migration of juvenile anemonefish from nurseries to breeding habitat.

## **Introduction**

Coral reefs are the most biodiverse marine habitats on Earth (Loya 1972, Jackson 1991), yet they are in precipitous decline, threatened by an increasing number of anthropogenic influences that include overfishing, nutrient loading, and climate change, among other factors. These processes are causing coral reefs in many parts of the world to support ever-decreasing levels of biodiversity (Wilson et al. 2006, Carpenter et al. 2008, Wilkinson 2008). In addition to stony corals and their contribution of hard structure to the reef, soft-bodied cnidarians such as soft corals and sea anemones play an integral role in coral reef ecosystems, especially through their mutualistic relationships (Haywood and Wells 1989, Sheppard et al. 2012, Fautin and Allen 1997, Nakano and Fujii 2014).

Giant sea anemones on coral reefs are vulnerable to population declines caused by anthropogenic stressors such as overfishing (Shuman et al. 2005) and temperature-induced bleaching (Hill and Scott 2012, Hobbs et al. 2013). Current threats to populations of reef anemones are especially worrisome due to the high diversity of fishes and crustaceans that depend on them as obligate symbiotic hosts (Bruce 1976, Patton 1976, Chadwick et al. 2008).

One of the most recognizable symbioses in nature is the partnership between clownfishes (anemonefishes) and giant sea anemones (Fautin and Allen 1997), but both organisms are decreasing in abundance due in part to being widely collected for the aquarium trade. Ten years ago, Shuman et al. (2005) reported that clownfish anemones were being harvested at rates that cannot be matched by their reproduction, and called for harvest rates to be adjusted to avoid further population declines and local extinctions. In addition to overharvesting, giant coral reef sea anemones are potentially affected by environmental stressors such as rising sea water temperature, habitat destruction, sedimentation, pollution, and local nutrient enrichment that contribute to reef degradation (Szmant 2002, Thornhill 2012). The effects of these various stressors on anemone populations and their associates remain widely unknown, other than the negative impacts of temperature-induced anemone bleaching (Jones et al. 2008, Hill and Scott 2012, Hobbs et al. 2013, Hill et al. 2014). Conversely, ocean acidification, while foundationally detrimental to coral reefs, may actually enhance the growth rates of some non-calcifying cnidarians, including symbiotic sea anemones (Suggett et al. 2012, Jarrold et al. 2013).

Little is known about the population dynamics of clownfish sea anemones. Several studies have documented their patterns of abundance (Hirose 1985, Hattori 1994,

Richardson et al. 1997, Elliott and Mariscal 2001, Brolund et al. 2004, Chadwick and Arvedlund 2005, Hattori 2006, Scott et al. 2011, Bridge et al. 2012, Huebner et al. 2012, Dixon et al. 2014, Scott and Baird 2014, Lee et al. 2015), sexual reproductive cycles (Chia 1976, Scott and Harrison 2007a, 2009; Bi et al. 2015), larval settlement and juvenile development (Scott and Harrison 2007b, 2008), as well as short-term changes in body size (Porat and Chadwick-Furman 2004, Holbrook and Schmitt 2004, 2005). However, no information is available on their long-term patterns of recruitment, growth, and mortality in the field. This is due in part to the challenges of applying standard age-based demographic models, because it is difficult to determine individual age and size-age relationships in sea anemones.

The demographic patterns of cnidarians with hard skeletons, such as some stony corals (Chadwick-Furman et al. 2000, Goffredo et al. 2004), and with flexible protein skeletons, such as branching soft corals (Goffredo and Lasker 2008), have been analyzed using age-based models from information in their skeletal growth rings. In contrast, sea anemones possess only soft tissues with no growth rings, and are able to not only grow but shrink. As such, their body size is easier to determine than their age (Chomsky et al. 2004, O'Reilly 2015). This pattern is similar to that in certain stony corals that fission or fusion, do not leave clear evidence of previous size (Hughes and Jackson 1985, Edmunds and Elahi 2007), and which have been analyzed using a size-based demographic model (Hughes 1984) modified from Leslie's (1945) classic age-based model. This type of model also can be applied to sea anemones. Information gained through this type of demographic analysis of giant sea anemones can determine the processes contributing to the current status of their populations, and is important for the projection of future trends

to serve as a basis for management decisions. In addition, the relative influence of natural versus anthropogenic environmental factors in controlling cnidarian populations on coral reefs often can be discerned from their demographic patterns (Bak and Meesters 1998, Chadwick et al. 2000, Guzner et al. 2010).

Understanding of sea anemone demography also is important due to their relevance to the fields of medicine and agriculture, through application of their nematocyst venoms and injection mechanisms. Sea anemones deliver highly toxic venom to potential enemies and prey via sophisticated subcellular capsules (nematocysts, Shick 1991). In the field of medicine, the cytolytic properties of giant sea anemone venom can be applied to fight various human diseases. Sea anemone venom kills lung, breast, and skin cancer cells (Ramezanpour et al. 2012) via targeted cell apoptosis (Ramezanpour et al. 2014). Venom from the sun anemone *Stichodactyla helianthus* inhibits a specific voltage-gated potassium channel that regulates basal metabolic rate in mice, suggesting potential as an anti-obesity drug for humans (Upadhyay et al. 2013). The venom delivery system of sea anemone nematocyst capsules also potentially can be loaded with medications, and function as a micro-injector for precise transdermal delivery of pharmaceuticals to injured tissues (Ayalon et al. 2011).

In agricultural systems, the venoms from sea anemone nematocysts are effective in the control of aphid populations in florist's chrysanthemum, which is the world's second most important floricultural product, next to roses (Valizadeh et al. 2013). Western flower thrips *Frankliniella occidentalis* feed on many commercial crops, and can potentially be controlled with multi-domain biological control that includes sea anemone venom (Outchkourov et al. 2004). Sea anemone venom can also help protect potato crops

against larval Colorado potato beetles *Leptinotarsa decemlineata*, which feed on potato plant leaves (Gruden et al. 1997). These novel venom applications employ sea anemone equistatin, a protease inhibitor produced by beadlet sea anemones *Actinia equina* which negatively affects insect survivorship and reproduction. This natural insecticide is environmentally-friendly because it breaks down into non-toxic byproducts that do not persist in ecosystems, in contrast to many synthetic pesticides. The industrial potential of these sophisticated venoms and nematocyst capsule delivery systems indicates the importance of understanding sea anemone demographic processes, so that their dwindling populations can be preserved.

Ten species of giant sea anemones host 30 species of obligate anemonefishes on Indo-Pacific coral reefs (Fautin and Allen 1997, Allen et al. 2008, Allen et al. 2010). This symbiotic relationship is mutually beneficial in that the anemonefish enhance host anemone growth and survivorship (Holbrook and Schmitt 2005, Wu et al. 2015) by providing nutrients (Roopin et al. 2008, Cleveland et al. 2011), protection from predators (Godwin and Fautin 1992, Porat and Chadwick-Furman 2004), and oxygenation (Szczebak et al. 2013). In turn, the abundance of anemonefishes depends on the abundance and species diversity of host anemones (Richardson 1999, Elliott and Mariscal 2001, Huebner et al. 2012). Anemonefishes must successfully locate a host to have a chance of survival; juvenile anemonefish are almost certainly preyed upon if unsuccessful (Fautin and Allen 1997). Because space for juvenile fish to recruit to a suitable host is limited, declines in host populations result in more limited habitat for anemonefishes. Thus, steep declines in anemonefish abundance will occur where anemones are overharvested or are in decline due to other factors.

On shallow coral reefs in the northern Red Sea, bulb-tentacle sea anemones *Entacmaea quadricolor* (Rüppell and Leuckart, 1828) and leathery sea anemones *Heteractis crispa* (Ehrenberg, 1834) host endemic two-band anemonefish *Amphiprion bicinctus* (Rüppell, 1828). Adult fish occur mainly in individuals of *E. quadricolor*, where they form breeding pairs and produce offspring. Juvenile fish are relegated mostly to individuals of the nursery anemone *H. crispa*, where they wait for openings in nearby *E. quadricolor*, and rapidly immigrate when vacancies arise (Huebner et al. 2012). As a result, the demographic patterns of these anemonefish are strongly influenced by host species identity and their dispersion patterns, as well as by host population dynamics.

I employ here a size-based demographic model (Hughes 1984) to analyze the dynamics of *E. quadricolor* and *H. crispa* over 4 years on a coral reef in the northern Red Sea at Eilat, Israel. I document patterns of recruitment, growth, shrinkage, stasis, and mortality of individuals, estimate rates of population turnover, and project future population trends for both anemone species. Based on these analyses, I provide the first scientifically-based recommendations for the sustainable harvest of these giant sea anemones on coral reefs.

## **Methods**

### ***Study site and collection of field data***

Annual field surveys were conducted each June for 4 years (1996-2000) on coral reefs adjacent to the Interuniversity Institute for Marine Science, at Eilat, Israel, northern

Gulf of Aqaba, Red Sea (29°30'04.00"N; 34°55'00.50"E). A study site (Fig. 2.1) extending 220 m x 50 m on the reef slope (long axis parallel to shore) was selected for population studies, because it contained enough individuals of bulb-tentacle sea anemones *E. quadricolor* and leathery sea anemones *H. crispa* for demographic analysis (> 70 individuals per species, after Ottaway 1980, Hirose 1985, Hattori 2006). The site ranged in depth from 0-12 m, and consisted of small patch reefs interspersed with sand and occasional large reef knolls (see detailed site description in Chadwick and Arvedlund 2005). Initially the site was mapped and thoroughly searched for sea anemones; each individual of the 2 species was marked by attaching an engraved aluminum tag to the adjacent reef substrate (after Porat and Chadwick-Furman 2004, Huebner et al. 2012; see details below on tagging and individual identification).

During June 1996, 88 individuals of *E. quadricolor* and 108 of *H. crispa* occurred inside the mapped site and were tagged. The following data were collected for each tagged sea anemone during each year of study: species, tag number, tentacle crown length and width, depth below sea surface, substrate type (rock or sand), orientation (vertical or horizontal orientation of the oral disk), number and size (total length, TL) of all associated twoband anemonefish *A. bicinctus*, and sea anemone markings to aid in individual identification (tentacle morphology after Huebner et al. [2012], striations on the oral disk or tentacles, presence of column vesicles, etc.). The largest distance across the center of the tentacle crown from tentacle tip to tentacle tip ( $L$  = length) and perpendicular across the center of the tentacle crown ( $W$  = width) were recorded, and TCSA was calculated using the equation for the area of an oval,  $\frac{1}{2}$  length x  $\frac{1}{2}$  width x  $\pi$  (after Hirose 1985, Hattori 2002, 2006, Brolund et al. 2004, Chadwick and Arvedlund

2005, Huebner et al. 2012). The sampling interval was once per year, after initial sampling every 3 months revealed only slight population changes during shorter periods. Each year, anemones that appeared contracted (tentacles and oral disk not fully expanded, Porat and Chadwick-Furman 2004) were re-examined during later dives in the same week, until the anemone oral disk appeared to be fully expanded and then re-measured; if they did not re-expand, they were marked as contracted, and their body size data were interpolated for that census year based on their body size during the previous and subsequent surveys. The entire study site was examined carefully each year, including all reef cracks and crevices, and any new sea anemones of the above 2 species were marked and measured.

### *Data analysis*

Changes in the populations were analyzed using a size-based demographic model (Fig. 2.2), in which individuals were grouped into classes based on body size, and transitions between the classes were determined each year (after Hughes 1984, Edmunds and Elahi 2007). Each individual was assigned to 1 of 3 size classes based on TCSA: I (< 150 cm<sup>2</sup>), II (150-300 cm<sup>2</sup>), or III (> 300 cm<sup>2</sup>). Transition matrices were constructed based on these size classes and on the fate of each individual during each year of the study.

During each annual survey after 1996, new individuals in the study site that were < 200 cm<sup>2</sup> TCSA were considered to be recruits, based on preliminary analysis of the maximum annual growth rates of recruits at Eilat (N. E. Chadwick, unpublished data).

These individuals were not considered strictly as recent settlers, rather as juveniles that had survived following settlement during the year prior to observation (after Hattori 2006). At Okinawa, Japan, where individuals of *H. crispera* grew to larger sizes (many > 1,000 cm<sup>2</sup>, and some > 2,000 cm<sup>2</sup>) than observed in the northern Red Sea, Hattori (2006) used a cutoff of < 500 cm<sup>2</sup> TCSA for anemone recruits. In accordance with smaller maximal body size and slower growth at our site, newly-tagged individuals with a TCSA > 200 cm<sup>2</sup> were assumed to be anemones that had been present in previous years but went undetected, and were excluded from analysis (3% of *E. quadricolor*, N = 133 over 4 years; 8% of *H. crispera*, N = 183). Small cnidarian recruits are notoriously difficult to locate on the convoluted substrates of complex reef systems (Grigg 1984, Chadwick-Furman et al. 2000, Goffredo and Lasker 2008); I thus did not classify as first-year recruits any newly-tagged anemones if they exceeded 200 cm<sup>2</sup>, and excluded them from analysis because they likely had been undetected during previous census samples.

Because these population transition matrices did not include the effects of sexual reproduction on population growth rate (due to unknown fecundity rates), the intrinsic rates of population growth ( $\lambda$ ) potentially could be misrepresented. To test effects of within-matrix reproduction rates on elasticity, supplemental transition matrices were constructed in which recruitment rates (i.e., the contribution of each size class to observed recruitment, see below) were calculated and summed directly with the transition elements in each cell. These supplemental matrices were constructed to test my assumptions concerning the reproductive output of these species, and to determine whether recruitment substantially affected matrix elasticity. All elasticity tables were calculated in Microsoft Excel 2013 using the PopTools add-in (version 3.2.5).

I used the following information to estimate the sexual reproductive contribution of each size class of resident sea anemone to recruitment: (1) Giant sea anemones on coral reefs mature sexually at very small body sizes: ~1 cm lower column diameter near the pedal disk in the Caribbean corkscrew anemone *Bartholomea annulata* (Jennison 1981), and ~2 cm or smaller pedal disc diameter (PDD) in *E. quadricolor* (Scott and Harrison 2009), roughly equal to 30 cm<sup>2</sup> TCSA ( $TCSA = 7.78[PDD]^{1.93}$ ,  $R^2 = 0.48$ ,  $N = 12$ , based on measurement of individuals under laboratory conditions). Thus, we assumed that 30 cm<sup>2</sup> TCSA was the minimum body size at sexual maturity for both species. Therefore, some individuals in Size Class I (4.1-149.5 cm<sup>2</sup> and 12.2-149.6 cm<sup>2</sup> TCSA for *E. quadricolor* and *H. crispa*, respectively) likely contributed sexual propagules to recruitment (~10% of all recruits, see below). (2) Sexual reproductive output (gonad volume, egg abundance, etc.) increases roughly linearly with body size in all species of actinian sea anemones examined to date, including a temperate intertidal brooder (Dunn 1977), broadcast spawners in the temperate intertidal (Sebens 1981, Bucklin 1982) and subtidal zones (Wedi and Dunn 1983), and on tropical coral reefs (Jennison 1981), as well as in Red Sea corallimorpharians (Chadwick-Furman et al. 2000). Thus, I assumed that individuals of *E. quadricolor* and *H. crispa* (both broadcast spawners, Scott and Harrison 2007a) increased their sexual reproductive output (number of propagules released) linearly with body size. (3) Based on the above reproductive patterns, the contribution of each size class of anemones here to recruitment likely was proportional to their body size; anemones in the largest size class (which included a few very large individuals) probably were responsible for the majority of recruits, while the smallest size class contributed little (though still some) to recruitment. I thus estimated the

proportional contribution to recruitment by each size class as I: 10%, II: 30%, and III: 60%. These proportional contributions corresponded to very similar proportional relationships among the mean body sizes of each size class (I = 71 cm<sup>2</sup> TCSA, II = 214 cm<sup>2</sup>, III = 472 cm<sup>2</sup>; thus mean size in I was ~ 1/3 the mean size in II, and that in II was ~ 1/2 the mean size in III).

Mortality rates were estimated based on the disappearance of anemones between census years. The study site was examined thoroughly each year, and anemones that could not be relocated were considered dead or lost to the population (after Ottoway 1979, Holbrook and Schmitt 2005). Although sea anemones are capable of limited motility (Mariscal 1972, Mitchell 2003, O'Reilly 2015), substantial movement by individuals is rare. In previous anemone motility studies (Ottoway 1978, Batchelder & Gonor 1981), most individuals that changed location moved < 3 cm over 1-2 years, and the greatest distance traveled was 25-30 cm. In the giant sea anemone *H. magnifica* at Moorea, few individuals moved > 1 m in 3 years (Holbrook and Schmitt 2005), and only one individual of *H. crispa* out of 76 (1.3%) on Japanese reefs moved > 1 m over 1.5 years (Hattori 2006). At our study site, marker tags were attached close to anemones (< 5 cm), which were sparsely distributed throughout the site (< 1 individual per 100m<sup>2</sup>, Chadwick and Arvedlund 2005). As such, even if an individual moved > 1 m in 4 years, it would have been difficult to confuse it with another marked individual. On one occasion, an individual of *E. quadricolor* was found detached on the reef surface, and exhibited severely damaged tissues as if attacked by a predator (N.E. Chadwick, personal observation). In all other cases, individuals of both species appeared to remain immobile with their bases attached deep inside reef holes (as described in Fautin and Allen 1997).

Occasionally, anemones not relocated during 1 year were found again in a subsequent year. Previous survey data and notes were examined to determine whether, based on size, location, and individual markings (see above), the anemone could have been missed in that year's survey, possibly due to being contracted inside its reef hole (Huebner et al. 2012). In cases where anemones with absent data for 1 year appeared to be the same individual as in previous and subsequent years, body dimensions were interpolated to yield an approximate TCSA for the year of missing data (15% of all anemones).

Population turnover time ( $T$ ) was calculated as the reciprocal of turnover rate (after Odum 1983, Hanski 1999), and estimated as the projected time for complete replacement of individuals based on observations from one annual census to the next, using the equation:

$$T = \left( \frac{M + R}{N_t + N_{t+1}} \right)^{-1}$$

where  $M$  = number of deaths (disappearances),  $R$  = number of recruits (appearances),  $N_t$  = number of individuals at the beginning of the annual period, and  $N_{t+1}$  = number that survived to the end of the annual period.

Elasticity analyses were applied to reveal which transition stages contributed the most to the intrinsic rate of growth ( $\lambda$ ). These stages were important to focus on for management because they can help identify the parts of the life history that contribute most to the growth of the population (Benton and Grant 1999).

Patterns of variation among years and between species in the size structure of the population, and in the proportion of individuals that recruited into or were lost from the

populations, were analyzed using Chi-square tests. Analyses of the other information that was collected for each sea anemone during annual surveys (see above), such as variation in the types of substrates they occupied, orientation of their oral disks, trends in body size with depth, and patterns of anemonefish association, are reported elsewhere (Wu et al. 2015). Results are reported here as means  $\pm$  one standard deviation unless noted otherwise.

## Results

### *Abundance and population structure*

At the beginning of the study in 1996, bulb-tentacle anemones *E. quadricolor* and leathery anemones *H. crispa* occurred at abundances of 0.080 and 0.098 individuals per 10m<sup>2</sup>, respectively (N = 88 and 108 individuals in the 220 x 50 m study site, Fig. 2.1). The abundance of individuals of *E. quadricolor* increased by 6% in 1997, then decreased by a mean of 11% each year for the next 3 years, resulting in a significant decline of 25% in 4 years, to only 0.060 individuals per 10m<sup>2</sup> in 2000 (N = 66 individuals, mean loss of  $6.3 \pm 3.4$  individuals per year, regression analysis,  $p < 0.05$ ,  $R^2 = 0.85$ , Fig. 2.3). The abundance of individuals of *H. crispa* remained stable from 1996-1998, then decreased by 15% in 1999, with a slight increase in the final year of study, resulting in a non-significant decline of about 14% in 4 years, to 0.084 individuals per 10m<sup>2</sup> in 2000 (N = 92 individuals, mean loss of  $5.1 \pm 3.6$  individuals per year,  $p = 0.052$ ,  $R^2 = 0.76$ , Fig.

2.3). During all 5 census years, individuals of *H. crispera* were about one-quarter more abundant ( $27 \pm 8\%$ , range = 17-39%) than were those of *E. quadricolor* (Fig. 2.3).

The relative abundance of each size class of bulb-tentacle anemones decreased with body size during most census years; small individuals were relatively more abundant than were large ones (Fig. 2.4). This pattern was reversed during 1997, when the population grew in abundance and mean body size, so that most individuals belonged to the largest size class. Conversely, leathery anemones had a fairly even distribution of individuals among size classes during the early years of the study when their abundance also remained stable. The size structure then shifted to comprise mostly small individuals, after the population began to decline in 1999 (Fig. 2.4). The proportion of individuals in each size class varied significantly among years for both *E. quadricolor* ( $\chi^2 = 34.6$ ,  $p < 0.001$ ) and *H. crispera* ( $\chi^2 = 30.7$ ,  $p < 0.001$ ). The proportion of individuals within each size class differed significantly between the 2 anemone species only during the first year of study (1996:  $\chi^2 = 7.79$ ,  $p < 0.05$ ; 1997:  $\chi^2 = 3.05$ ,  $p = 0.22$ ; 1998:  $\chi^2 = 3.22$ ,  $p = 0.20$ ; 1999:  $\chi^2 = 0.37$ ,  $p = 0.83$ ; 2000:  $\chi^2 = 3.03$ ,  $p = 0.22$ ).

### ***Population dynamics***

The smallest size class changed more than did the larger size classes in all years for both anemone species (Table 2.1). Both recruitment ( $R_n$  in Table 2.1, which by definition occurred into only the 2 smallest size classes) and loss (number of individuals that died,  $q_x N$  in Table 2.1) occurred mostly among the smallest individuals in terms of absolute numbers, in all 4 years for both species. In contrast, medium-sized individuals

experienced intermediate levels of mortality and recruited more rarely, and members of the largest size class were rarely lost from the population (experienced low mortality), in terms of absolute numbers ( $q_x N$  and  $R_n$ ). Proportionally, the mean loss rate of individuals from the largest size class was 9% ( $\pm 8\%$ ) for *E. quadricolor* and 8% ( $\pm 2.5\%$ ) for *H. crispa*.

For bulb-tentacle anemones, the probability of mortality was high in the largest size class during the final year of the study, when only 9 individuals remained in the largest size class ( $q_x$  in Table 2.1A). For leathery anemones, mortality rate was roughly equivalent in the 2 smaller size classes and lowest in the largest size class for all years, except during the final year of study when it was highest among medium-sized individuals.

Mortality rates for both species fluctuated from year to year; neither species stood out as regularly having the highest mortality. The greatest disparities between the 2 species occurred during the last 2 years, when *E. quadricolor* mortality was less than half that of *H. crispa* in 1999, and then in 2000 *H. crispa* mortality rate was just over half that of *E. quadricolor* (Fig. 2.5B). Mean annual mortality rate from 1996-2000 was 0.18 for *E. quadricolor*, and 0.20 for *H. crispa*, indicating a loss of about 1/5 of the population to mortality each year. During the first year, recruitment outweighed mortality causing a slight population increase for both species, but subsequently this pattern reoccurred only once, when *H. crispa* recruitment eclipsed mortality in the final transition year. The *E. quadricolor* population declined at a slightly more rapid rate than did that of *H. crispa*, leading to abundances of only 75% and 86% of their original population sizes after 4 years, respectively.

For bulb-tentacle anemones, during the first year the proportion of individuals that remained static (in the same size class between years) varied widely with body size, from only 17% of medium-sized to 74% of large individuals (Table 2.1A). However, during subsequent years a more even proportion of individuals remained static, ranging from 26-67% of individuals, depending on size class. Leathery anemones exhibited more stasis, as a majority of the individuals in each size class remained static during most years (Table 2.1B).

Across all years combined, stasis accounted for the largest transitional property in both species. On average, 42% of individuals of *E. quadricolor* in each size class remained static each year (range = 17-74% of individuals, Table 2.1A), similar to the proportion in *H. crispa* (49%, range = 24-78% of individuals, Table 2.1B). Anemones that shifted into a smaller size class (shrank) made up the next largest transitional property for both species. From 1996-2000, an average of 37% (range = 12-64%) and 27% (range = 5-72%) of individuals of *E. quadricolor* and *H. crispa* shrank respectively; shrinkage occurred mostly in the largest size class for both species, except during the final transition year (1999-2000).

In terms of among-year variation, a high proportion of *E. quadricolor* individuals grew (transitioned into larger body size classes, 45-67%) and relatively few shrank (only 12-21%) during the first year when population abundance also increased (compare Table 2.1A and Fig. 2.3). During subsequent years as abundance declined, few individuals grew (only 5-28% depending on size class and year), while many shrank (22-64%), especially among the largest anemones during years 2 and 3. Slightly less shrinkage and more

growth occurred in year 4 than in years 2 and 3, indicating a slight upswing in population parameters for this species near the end of the study.

Among-year changes for *H. crispa* differed somewhat from those for *E. quadricolor*. During the first few years while population abundance was stable, a large proportion of *H. crispa* individuals grew (22-36%) and relatively few shrank (5-35%). Then, very few grew (only 7-9% of individuals) and a high proportion shrank (32-72%) during year 3 when abundance declined. Finally in year 4, the proportion that grew increased somewhat (8-32% of individuals) and fewer shrank (11-16%), as population abundance increased slightly (compare Fig. 2.3 and Table 2.1B).

Individuals of *E. quadricolor* in Size Class I exhibited the most rapid growth during the first transition period, with 45% reaching a subsequent size class (compared to 23% for *H. crispa*). In the following years, they grew less, until a slight uptick in 2000. Growth of *H. crispa* out of the smallest size class was most rapid during the final transition period (32%), though cumulatively individuals advanced into larger size classes more quickly than *E. quadricolor* (Table 2.1B). The majority of individuals in Size Class III remained static during the first and final transition periods for *E. quadricolor*, and in all but 1 year for *H. crispa* (year 3). The average proportion of stasis in this size class was lower for *E. quadricolor* (49%) than for *H. crispa* (57%). In periods where stasis was reduced, shrinkage into to smaller size classes was the dominant fate. Average mortality in Size Class III was low and similar between species, although *H. crispa* exhibited a slightly lower (1% less) mean mortality rate than did *E. quadricolor* among large individuals.

Individuals of *E. quadricolor* that survived all 4 years (N = 45) revealed overall rates of change in body size that were highly variable within each initial size class, but still varied significantly among the 3 size classes (ANOVA,  $F = 23.18$ ,  $p < 0.001$ ; Size Class I:  $100.0 \pm 149.7 \text{ cm}^2$  per year, II:  $33.5 \pm 163.6 \text{ cm}^2$ , III:  $-111.4 \pm 209.8 \text{ cm}^2$ , i.e., net shrinkage, Fig. 2.6A). These absolute size changes corresponded to even more dramatic differences in percent size change among the 3 initial size classes. The smallest anemones more than doubled their body size each year (I:  $216.8\% \pm 393.5\%$ ), medium anemones grew only slightly each year on average (II:  $17.3\% \pm 78.0\%$ ), and large anemones lost about a quarter of their tentacle crown surface area each year ( $-23.7\% \pm 48.5\%$ , Fig. 2.6B).

In the 48 leathery anemones that survived all 4 years, overall rates of change in body size also showed high variability within each initial size class, but again varied significantly with size class (ANOVA,  $F = 8.77$ ,  $p < 0.001$ ; Size Class I:  $46.2 \pm 121.6 \text{ cm}^2$  per year, II:  $14.2 \pm 109.6 \text{ cm}^2$ , III:  $-66.6 \pm 216.8 \text{ cm}^2$ , i.e., net shrinkage, Fig. 2.7A). These absolute size changes corresponded to smaller variation in percent size change among the 3 initial size classes, than was observed for *E. quadricolor*. The smallest individuals of *H. crispa* grew by about 50% each year (I:  $51.6\% \pm 114.3\%$ ), medium anemones grew very little (II:  $8.9\% \pm 52.4\%$ ), and large anemones lost  $< 20\%$  of their tentacle crown surface area each year ( $-16.6\% \pm 44.3\%$ , Fig. 2.7B).

Overall population shrinkage ( $\lambda < 1$ ) occurred in both species, where  $\lambda$  ranged from 0.69 to 0.93 (mean = 0.81) for *E. quadricolor*, and from 0.68 to 0.85 (mean = 0.79) for *H. crispa*. While  $\lambda$  fluctuated among years for both species, the final year of the survey yielded values of 0.77 and 0.83 for *E. quadricolor* and *H. crispa*, respectively. At

these rates, the populations of both species were expected to continue to decline. These values excluded population growth through reproduction, because fecundity was not included in the matrix; thus they describe only the rate of decline in these populations. Elasticity analysis revealed that  $\lambda$  was most elastic (proportionally sensitive) to stasis within Size Class III in years 1 and 4 (when both populations were mainly stable or grew), whereas in years 2 and 3 (when both populations shrank substantially)  $\lambda$  was most elastic to stasis within Size Class I for both species (except for *H. crispa* in year 2, where the vital rate that  $\lambda$  was most elastic to was split with stasis in Size Class III, Table 2.1).

Supplemental transition matrices (Table 2.2) that showed the effects of recruitment rates within the transition elements allowed for comparison with the original transition matrices, to determine any major effect of reproduction on elasticity. Elasticity values derived from the supplemental matrices that included recruitment rates did not differ (compared to values in the matrices that excluded recruitment rates), in terms of which matrix elements  $\lambda$  was most elastic to, for any of the transition periods in either species (Compare Table 2.3 and Table 2.4).

Leathery anemones recruited on average twice as frequently to the population as did bulb-tentacle anemones (range = 33% to 175% more recruits per unit area, depending on the year, Fig. 2.3). Recruitment declined substantially in both species during the first several years of the study, then increased during the final year, in parallel with a slight increase in body growth and decrease in shrinkage for both species. A higher percentage of individuals recruited (entered the population, Fig. 2.5A) than died (were lost to the population, Fig. 2.5B) during the first year for both species, then the pattern reversed so

that mortality was higher than recruitment, leading to an overall trend of decline in population abundance (Fig. 2.3).

The total number of anemones that recruited from 1996-2000 was 106: 39 *E. quadricolor* and 67 *H. crispa*. New recruits made up on average 13% and 17% of the population of *E. quadricolor* and *H. crispa* each year, respectively, and recruitment was highest in the first and final transition periods for both species (Fig. 2.3). Thus, on average these populations gained less than 1/5<sup>th</sup> of their individuals from recruitment each year. Following decreasing rates of recruitment in 1998 and 1999, recruitment success peaked for both species in 2000, when 20% of the *E. quadricolor* population and 22% of the *H. crispa* population consisted of new recruits. During their first year on the reef, almost all recruits of *E. quadricolor* either remained in Size Class I (46%) or died (46%), with the remaining 8% growing into larger size classes. Recruits of *H. crispa* were more dynamic during their first year, with 40% dying, 36% remaining in Size Class I, 13% transitioning to Size Class II, and 11% advancing all the way to Size Class III. Only a small proportion of individuals (13%, N = 15) of *E. quadricolor* that recruited in 1997 survived to 2000, while almost 3 times as many (35%, N = 20) *H. crispa* recruits from 1997 survived to 2000. Thus, individuals of *H. crispa* overall produced relatively more recruits per year, which survived longer, and grew more, than did those of *E. quadricolor*.

Taken together, the above patterns revealed that both populations were highly dynamic, with turnover times of approximately 7 and 5 years for bulb-tentacle and leathery anemones, respectively (Table 2.5).

## Discussion

Coral reefs at Eilat have been in decline for the past half century, but the pace of degradation and the total loss in organismal abundance during that time remains mostly unquantified (Loya et al. 2004, Rinkevich 2005). The demographic patterns reported here elucidate aspects of this decline in the 2 most common sea anemone species on these reefs for a portion of this 50-year timespan. Our analysis revealed patterns of low recruitment, body shrinkage, and relatively high mortality in both species, which contributed to alteration of population structure and to a steady decline in the abundance of both anemones over 4 years. This decline has continued at about the same rate during the first 15 years of the 21st century, as that observed here in the late 1990s, leading to a currently very low abundance of both anemone species in this Israeli reef area (Chapter 3).

The population dynamics of these 2 species of giant sea anemones appeared to be linked, in that they experienced parallel changes in abundance except during the final year of the present study, when *H. crispa* abundance rose and *E. quadricolor* abundance declined, despite an uptick in recruitment. However, only one population of each species was examined, so it was not possible to statistically test this idea. Their observed per-year rates of decline in abundance (average losses of 6.3 and 5.1 individuals per year for *E. quadricolor* and *H. crispa*, respectively), when extrapolated into the future, yielded a pattern in which *E. quadricolor* numbers were projected to reach zero approximately 8 years before those of *H. crispa* (in 2010 vs. 2018, see Chapter 3). Although the rate of decline observed here over 4 years was greater in *E. quadricolor* than in *H. crispa*, recent

surveys indicated that in 2014, both populations are still in decline, with few individuals left at this study site, but that the between-species difference was reversed (Chapter 3). Over the past 18 years (1996-2014) at this study site, individuals of *E. quadricolor* have declined by 59% and of *H. crispa* by 83%, to a low of 36 and 18 individuals remaining, respectively (Chapter 3).

Individuals of *E. quadricolor* are the preferred hosts for the endemic Red Sea anemonefish *A. bicinctus*, and the only local host in which these fish are able to breed (Huebner et al. 2012). As the abundances of both hosts have dwindled at Eilat over the past 2 decades, individuals of *E. quadricolor* have steadily acquired a concentration of ever-larger fish associates relative to *H. crispa* (i.e., fewer, larger fish concentrating in *E. quadricolor* relative to the small individuals possible in *H. crispa*, Chapter 3). These larger anemonefish probably contributed more nitrogen (Roopin et al. 2008), oxygenation (Szczebak et al. 2013), and protection (Porat and Chadwick-Furman 2004) to their *E. quadricolor* hosts than did the much smaller fishes in *H. crispa*, thus perpetuating slightly higher abundances of the remaining *E. quadricolor* (Chapter 3) than projected here. Other density-dependent processes also may have affected the rates of decline of these populations, in that as the anemones became rarer at this site, it may have become more difficult for predators to locate and consume them. As such, the rates of decline for these anemones may have become slower as the individuals became sparser.

The patterns of declining abundance observed here are similar to those recorded in numerous studies of reef coral demography around the world, in which once-common coral species have experienced significant declines over recent decades. Hughes and Tanner (2000) observed substantial declines in the percent cover, survival, growth, and

recruitment of the 3 common Caribbean stony corals *Montastraea annularis*, *Agaricia agaricites*, and *Leptoseris cucullata* on the north coast of Jamaica over 16 years (1977-1993). In the U.S. Virgin Islands, abundances of the reef-building coral *M. annularis* experienced a 57% decline from 1988-2003 (Edmunds and Elahi 2007). A similar drastic decline in clownfish sea anemone abundance was observed in Japan following a bleaching event in 1998, in which the anemones declined by an average of 88.4% (Hattori 2002). Stony corals at Eilat also historically have experienced sharp decreases in abundance: 10 common coral species declined by an average of 77% over 4 years from 1969 to 1973 at the nature reserve near our study site, following an extreme low tide (September 1970), where post-disaster recruitment was thought to be inhibited by local phosphate eutrophication and chronic oil pollution (Loya 1975). Numbers of coral colonies on the Eilat reef flat have generally increased since the early 1970s, but remain well below their pre-low tide abundances (Wielgus et al. 2003). Unfortunately, declines in reef-building corals impact the entire reef ecosystem through their effects on reef growth rates and on the myriad organisms that depend on living coral colonies for food, habitat, and other services.

The long-term loss of giant sea anemone individuals from populations, as documented here, affects their obligate ectosymbionts in complex ways. Hattori (2002) observed the local extinction of a competitively superior anemonefish during host anemone decline, while an anemonefish that is a superior disperser was able to persist. As giant sea anemone populations continue to shrink in the Red Sea and around the world, the organisms that interact with them both directly and indirectly will likely also experience local to regional extinctions, as the available habitat that these reef hosts

provide diminishes. Especially worrisome is the loss of habitat for cleanershrimps that are obligate symbionts of giant sea anemones, because these shrimps provide major cleaning services by removing parasites that affect a wide diversity of reef fishes (McCammon et al. 2007, Chadwick et al. 2008, Huebner and Chadwick 2012, Titus et al. 2015).

The population size structure of *E. quadricolor* here followed a conventional distribution, with the greatest abundance of individuals in the smallest size class, and decreasing with body size. The only year this size structure trend differed was in 1997, when individuals in the largest size class dominated and reached their highest abundance of the study, corresponding also to the period with the highest number of recruits. Although the *H. crispera* population exhibited different size structure patterns, the number of individuals in the largest size class peaked along with *E. quadricolor* in 1997, which also corresponded with their highest recruitment (tied with 2000). Not surprisingly, the annual period that exhibited the greatest abundance of large individual anemones paralleled the period with the combined highest recruitment. These results support the idea that reproductive fitness (and local recruitment rate) in these anemones correlates with adult body size, as known for other anemones and corallimorpharians (Dunn 1977, Sebens 1981, Jennison 1981, Bucklin 1982, Wedi and Dunn 1983, Chadwick-Furman et al. 2000). Because recruitment rates may depend in part on the local abundance of large anemones due to self-recruitment of larvae to their home reefs (Nelson 2008), local population density plays a major role in the abundance of new cnidarian recruits on reefs (Hughes et al. 2000, Glassom et al. 2004). Thus in these anemones, a potentially high

level of self-recruitment to the population is likely, based on the similar patterns known for other demersal reef organisms (Swearer et al. 2002).

As expected, in most years mortality rate was highest in the smallest size class for both species. The largest size class for both species exhibited comparatively little mortality, indicating a possible size refuge from mortality in these anemones. However, mortality rate of all size classes increased overall during the study, indicating that even large individuals may experience high mortality and shrinkage (Fig. 2.5B). Rapid population turnover appeared to result mainly from the high mortality and regular incoming recruitment in both species, resulting in the replacement of most individuals every few years (Table 2.5). In terms of the factors that affect mortality rates in clownfish anemones, the number and size of hosted fish per anemone appear to be major predictors. In French Polynesia, individuals of *H. magnifica* that died or shrank during a 3-year study mostly lacked resident anemonefish (Holbrook and Schmitt 2005). At our study site, very few of the medium-sized individuals of *E. quadricolor* that each hosted 1-2 fish died over 4 years, while those with no fish had much higher rates of shrinkage and mortality (Porat and Chadwick-Furman 2004). An analysis of the environmental factors influencing body size in *E. quadricolor* and *H. crispa* at Eilat, including the presence of symbionts, revealed that the main predictor was the size of resident anemonefish. Other factors, such as fish number, microhabitat type, and oral disk orientation, contributed little to predicting rates of body size in both species (Wu et al. 2015). In addition to preventing anemone shrinkage by providing excreted nutrients (Roopin and Chadwick 2009, Cleveland et al 2011) and ventilation services (Szczebak et al. 2013), anemonefish play a major role in host growth and survival by defending them from predators. Individuals of

*E. quadricolor* at Eilat that lack anemonefish are attacked and preyed upon by diagonal butterflyfish *Chaetodon fasciatus* (Chaetodontidae), causing them to contract their oral surface, which prevents light exposure and effectively halts endosymbiotic photosynthesis (Porat and Chadwick-Furman 2004). Sea turtles also prey on the soft tissues of these sea anemones in the Red Sea. Multiple anemonefish in nearby Jordan have been observed to attack the eyes of sea turtles passing through the reef, until the turtle is out of the area (L. Huebner, personal communication). On a reef in the Egyptian Red Sea, divers recently observed an entire individual of *E. quadricolor* (with no anemonefish present) to be ripped off the reef and consumed by a hawksbill turtle (K. Tkachenko, personal communication). Taken together, this information indicates the central importance of both the size and number of resident anemonefish to host anemone growth and survival, and consequently the need for careful management of anemonefishes in order to prevent local extinction of hosts.

The pattern observed here that the highest average transition probabilities were for individuals remaining in the same size class (stasis), is similar to that in 2 species of Caribbean scleractinian corals in which most individuals remained static among years, and did not transition among size classes (supplemental data in Edmunds 2010). Levels of stability in both the anemone populations were related to their patterns of growth and shrinkage. When larger proportions of individuals grew and fewer individuals shrank, abundance was high or increasing. Conversely, when few individuals grew and shrinkage increased, abundance declined. Many individuals experienced 1-2 years of body shrinkage prior to death. This pattern is similar to that observed in corkscrew sea

anemones in the Caribbean, in which individuals experienced gradual shrinkage before death (O'Reilly 2015).

For both species, the patterns of body size change in the anemones that survived all 4 years differed from that of all anemones including those who died or recruited into the populations, indicating contrasting influences on growth in long-surviving versus more ephemeral individuals. The much more rapid growth of the smallest individuals of *E. quadricolor* who survived all 4 years, than those of *H. crispa*, suggests that long-term survivorship of the former species is tied—more strictly than *H. crispa* is—to rapid growth when small. The slower growth of long-surviving individuals of *H. crispa*, and more extreme patterns of size changes when ephemeral, than in *E. quadricolor*, may reflect the benefits of hosting large fish in *E. quadricolor*. Large anemonefish may allow long-surviving individuals of *E. quadricolor* to grow faster, but also may cause relatively slower growth than in *H. crispa* in short-lived individuals who may not have very large fish. In short, *E. quadricolor* may be more highly variable in processes than is *H. crispa* because they depend more on their fish residents, which may vary widely in both number and size over time, as a major impact on their population trends.

Previous studies have shown that seasonality also influences the growth of clownfish anemones; in Moorea, individuals of *H. magnifica* grew mostly during the summer months when both daylength was longer and seawater temperature was higher than in winter (Holbrook and Schmitt 2005). Future studies could examine these types of seasonal trends in the 2 anemone species examined here, which were not detectable in the present study due to only once-per-year sampling.

Recruitment rate was higher in *H. crispera* than in *E. quadricolor*, and recruits of the former species both grew more rapidly into larger size classes and survived longer than did those of the latter. Thus *H. crispera* appears to be a more dynamic, “weedy” species that reaches a large body size (and thus probably also reproductive maturity) relatively quickly, and produces more offspring (recruits) than do individuals of *E. quadricolor*, but also experiences higher mortality and more rapid turnover. In the years during which both populations were relatively stable (transition years 1 and 4), the proportion of recruits was roughly the same for both species, with *H. crispera* having a slight edge. However, in the middle transition years that experienced population decline, *H. crispera* had nearly double the proportion of recruits of *E. quadricolor*. A relatively high reproductive potential of *H. crispera* is consistent with the findings of Scott and Harrison (2007a), in which *H. crispera* spawned over a longer period of time each year than did *E. quadricolor*, and also had up to twice the number of spawning events during that period. Although *H. crispera* had the potential to maintain stability during the middle transition years through an increased recruitment effort, their lack of population growth is likely due to the same factors that deteriorated their growth and stability among the other transition elements during that time. Nevertheless, even with lower reproductive potential, populations of *E. quadricolor* may be more stable and less likely to decline rapidly, due to their status as preferred hosts for anemonefish, in that they host larger fish which provide more benefits to maintain body size (Huebner et al. 2012, Wu et al. 2015).

The intrinsic rate of population growth ( $\lambda$ ) was most elastic to stasis in 2 transition elements: the largest and smallest size classes for both species. This was partially a result of stasis being the most prominent fate in both species for all years. In

transition years 1 and 4,  $\lambda$  was most elastic to stasis in the largest size class, whereas in transition years 2 and 4,  $\lambda$  was most elastic to stasis in the smallest size class. While this demographic pattern may be a function of a number of variables, it parallels the trend in recruitment (Fig. 2.3), which dropped markedly in years 2 and 3 for both species, before increasing in year 4. If recruitment is low in a given year, the increase (or decrease) of the population as a whole could be most elastic to the group that maintains individuals within the smallest size class, as that group receives fewer additional members to contribute to the future of the population. Conversely, in years where recruitment is elevated (e.g., transition years 1 and 4), an elasticity value that mostly depends on maintaining the largest individuals (viz., those that produce the most offspring) could mean that recruitment is at a healthy level and population growth depends mostly on those individuals that provide the greatest contribution to new recruits (i.e., Size Class III). Both elements—maintenance of the very smallest and largest individuals in the population—should be focused upon when developing a sustainable management plan for organisms with such dynamic transitional potentials.

Analysis of the supplemental matrices that added recruitment to the matrix elements produced no changes to the transition elements that  $\lambda$  is most elastic to. This shows that at least for these 2 sea anemone populations at Eilat, recruitment does not play a major role in elasticity, as  $\lambda$  is most proportionally sensitive to the strongest transition elements (e.g., stasis) for these species. Thus, we can be confident in our more parsimonious matrix analysis that excludes recruitment from the matrix, and instead includes it as a vector (after Fong and Glynn 1998, Hughes and Tanner 2000).

### ***Management recommendations***

The populations examined here at Eilat are legally protected from harvest, but information on their patterns of recruitment and population turnover can be used to inform the sustainable management of harvested populations in other parts of the world, where they are intensively collected for the aquarium trade (Wabnitz et al. 2003, Shuman et al. 2005, Scott and Baird 2014). In addition, management recommendations based on other studies can be made and collected together to provide a comprehensive set of guidelines for harvest of these species.

Rates and patterns of commercial harvest should not surpass the capacity of these sea anemones to replenish their populations (Shuman et al. 2005). Scott and Harrison (2007a) report that breeding periods for *E. quadricolor* and *H. crispa* are limited. They also observed that even during spawning events, individuals that spawned were sometimes a minority in the population. Because recruitment of these species is considered to be sporadic (Fautin and Allen 1997), concentrated efforts must be made to protect their limited breeding seasons in order to maximize potential gamete release. A seasonal harvest closure around the estimated spawning periods would allow reproducing individuals another annual chance to spawn before potential collection. Spawning periods for these species off the eastern coast of Australia encompass only a few nights annually between summer and fall, just after annual peak irradiance, and near the annual peak of seawater temperature (Scott and Harrison 2007a). Thus, a few-months closure of the anemone collection season around the period of annual peak seawater temperature may allow a spawning and recruitment window each year for mature individuals of this

species. An issue with a closed season is that local harvesters may receive higher pay for anemones than for anemonefish, and thus suffer more economically from a seasonal closure of anemone harvest; management practices need to consider carefully the socio-economic framework within each area where harvesting takes place (Shuman et al. 2005).

Another management option is to designate protected areas near the harvesting zones for coral reef sea anemones (Nelson 2008, Shuman et al. 2005), which would allow protected populations to replenish the nearby harvested areas without necessarily requiring a closed season, thus still permitting harvest upon demand. Scott et al. (2011) observed that a 5-fold increase of *E. quadricolor* over 14 years in eastern Australia was at least partially related to long-term protection within a marine park. Designated protected areas adjacent to (and preferably up-current from) harvested areas would allow higher survival of large adult sea anemones within the protected area, which then could supply more recruits locally to downstream reefs. In addition, protected areas could maintain the minimum abundances needed (i.e., nearest neighbor distances) to allow for adequate densities of spawned gametes, which are necessary for successful fertilization and larval production (Levitan 1991).

Establishing a minimum body size for harvest would further support a sustainable anemone fishery. Our observations indicated that the smallest size classes of both *E. quadricolor* and *H. crispa* have a prominent influence on the growth and overall numbers in the populations. This important contribution by small individuals is similar to the pattern for populations of the waratah anemone *Actinia tenebrosa* in New Zealand (Ottoway 1980) and the corkscrew anemone *Bartholomea annulata* in St. Thomas, U.S. Virgin Islands (Nelson 2008). The elasticity results also reveal the value of the largest

size class to population growth, especially during years when recruitment rate is low. Management directed at enhancing recruitment and retaining the largest individuals could be most effective to maintain sustainable populations of *E. quadricolor* and *H. crispa*. Preservation of the largest individuals would also protect the anemones in which mated pairs of anemonefish begin to breed. At our study site, pairs of adult *A. bicinctus* (defined as individuals  $\geq 6$  cm total length, after Fricke 1983, Huebner et al. 2012) typically inhabit the largest individuals of *E. quadricolor* (81% of breeding pairs observed from 1996-2014 were found in anemones larger than 200 cm<sup>2</sup>). Reproductive success in the anemonefish *A. percula* relates to the growth and size of females, which are based on host anemone size (Buston and Elith 2011). Thus, a slot limit—allowing the collection of only medium-sized individuals—could serve as a valuable tool to maintain local sustainable populations of sea anemones and their associates.

Finally, because *H. crispa* serves as a nursery species for *A. bicinctus* (Huebner et al. 2012), their preservation at locations near *E. quadricolor* individuals would help to sustain the anemonefish population, by maintaining habitat for the recruitment of juveniles waiting for an opportunity to emigrate to the preferred host *E. quadricolor*. The observed distances that *A. bicinctus* individuals travel to relocate to an *E. quadricolor* anemone may be  $< 5$  m (Huebner et al. 2012), although some anemonefish species can migrate up to 50 m (Hattori 1994).

In conclusion, I recommend a combination of at least some aspects of the following 4 measures, to ensure sustainable management of these 2 host sea anemones for an ornamental fishery: (1) Seasonal closure of harvest during their estimated annual spawning periods, to allow especially the largest individuals to release abundant gametes,

(2) Designation of nearby protected areas where anemones are not harvested, preferably up-current from harvested (or otherwise degraded) reefs, to support local recruitment and population replenishment, (3) A slot limit that allows collection of only medium-sized individuals, which have relatively little impact on population changes, and (4) Protection from harvest of interspecific pairs of hosts, in which nursery anemones *H. crista* are located < 5 m distant from anemones *E. quadricolor* that host breeding anemonefishes, to allow the migration of juvenile fishes from nurseries to breeding habitat.

Although they are not harvested in Israel, the continued population decline of these sea anemone species in Israel may be due to a complex array of anthropogenic factors that impact the reef systems as a whole (Loya et al. 2004, Rinkevich 2005). These include residual effects of nearby open-sea pen-net fish farms, intensive shoreline development leading to the release of various pollutants and changes in sedimentation on adjacent reefs, and intensive diving tourism, among other factors (Zakai and Chadwick-Furman 2002, Loya et al. 2004, Rinkevich 2005, Guzner et al. 2010). Based on their current rates of decline (Chapter 3), concentrated management efforts will be necessary to prevent the local extinction of these sea anemones and their obligate fish associates in Israel. Fortunately, populations across the Gulf of Aqaba in Jordan appear to be abundant and stable, due to a combination of mostly political but also probably oceanographic factors that differ on the opposite sides of this narrow gulf (McVay and Chadwick, in preparation). Research seeking to provide practical ways to offset declining populations of reef organisms with the high demand of the aquarium trade is increasing, yet more work is needed to prevent a collapse in reef invertebrate fisheries (Rhyne et al. 2009). Asexual propagation of giant sea anemones may have the potential to partially satisfy

high commercial demand, and also strategically to replenish reefs (Scott et al. 2014). Giant sea anemone harvest for the aquarium trade, coupled with other anthropogenically-related contributors to coral reef decline, should be counteracted with scientifically-based management, such as implementations of the recommendations made here, in order to preserve these iconic symbiotic coral reef hosts.

## References

- Allen, G.R., Kaufman, L., Drew, J.A. (2008) *Amphiprion barberi*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, and Samoa. *Aqua, International Journal of Ichthyology* 14(3):105-114.
- Allen, G.R., Drew, J., Fenner, D. (2010) *Amphiprion pacificus*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, Samoa, and Wallis Island. *Aqua, International Journal of Ichthyology* 16(3):129-138.
- Ayalon, A., Shichor, I., Tal, Y., Lotan, T. (2011) Immediate topical drug delivery by natural submicron injectors. *International Journal of Pharmaceutics* 419:147-153.
- Bak, R.P.M., Meesters, E.H. (1998) Coral population structure: the hidden information of colony size-frequency distributions. *Marine Ecology Progress Series* 162:301-306.
- Batchelder, H.P., Gonor, J.J. (1981) Population characteristics of the intertidal green sea anemone, *Anthopleura xanthogrammica*, on the Oregon coast. *Estuarine, Coastal and Shelf Science* 13(3):235-245.
- Benton, T.G., Grant, A. (1999) Elasticity analysis as an important tool in evolutionary and population ecology. *Trends in Ecology and Evolution* 14:467-471.
- Bi, Y., Zhang, B., Zhang, Z., Qiu, J. (2015) Seasonal Gametogenesis of Host Sea Anemone (*Entacmaea quadricolor*) Inhabiting Hong Kong Waters. *Journal of Ocean University of China* 14:143-148.
- Bridge, T., Scott, A., Steinberg, D. (2012) Abundance and diversity of anemonefishes and their host sea anemones at two mesophotic sites on the Great Barrier Reef, Australia. *Coral Reefs* 31(4):1057-1062.
- Brolund, T.M., Tychsen, A., Nielsen, L.E., Arvedlund, M. (2004) An assemblage of the host anemone *Heteractis magnifica* in the northern Red Sea, and distribution of the resident anemonefish. *Journal of the Marine Biological Association of the United Kingdom* 84:671-674.
- Bruce, A.J. (1976) Shrimps and prawns of coral reefs, with special reference to commensalism. *Biology and Geology of Coral Reefs* 3:37-94.
- Bucklin, A. (1982) The annual cycle of sexual reproduction in the sea anemone *Metridium senile*. *Canadian Journal of Zoology*, 60(12):3241-3248.

- Buston, P.M., Elith, J. (2011) Determinants of reproductive success in dominant pairs of clownfish: a boosted regression tree analysis. *Journal of Animal Ecology* 80(3):528-538.
- Carpenter, K., Abrar, M., Aeby, G., Aronson, R.B., Banks, S., Bruckner, A., et al. (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* 321:560-563.
- Chia, F.S. (1976) Sea anemone reproduction: patterns and adaptive radiations. In *Coelenterate ecology and behavior*. Springer, United States. pp. 261-270.
- Chomsky, O., Kamenir, Y., Hyams, M., Dubinsky, Z., Chadwick-Furman, N.E. (2004) Effects of feeding regime on growth rate in the Mediterranean sea anemone *Actinia equina* (Linnaeus). *Journal of Experimental Marine Biology and Ecology* 299:217-229.
- Cleveland, A., Verde, E.A., Lee, R.W. (2011) Nutritional exchange in a tropical tripartite symbiosis: direct evidence for the transfer of nutrients from anemonefish to host anemone and zooxanthellae. *Marine biology* 158(3):589-602.
- Chadwick-Furman, N.E., Spiegel, M., Nir, I. (2000) Sexual reproduction in the tropical corallimorpharian *Rhodactis rhodostoma*. *Invertebrate Biology* 119(4):361-369.
- Chadwick-Furman N.E., Goffredo, S., Loya, Y. (2000) Growth and population dynamic model of the reef coral *Fungia granulosa* Kluzinger, 1879 at Eilat, northern Red Sea. *Journal of Experimental Marine Biology and Ecology* 249:199-218.
- Chadwick, N.E., Arvedlund, M. (2005) Abundance of giant sea anemones and patterns of association with anemonefish in the northern Red Sea. *Journal of the Marine Biology Association of the United Kingdom* 85:1287-1292.
- Chadwick, N.E., Ďuriš, Z., Horká, I. (2008) Biodiversity and behavior of shrimps and fishes symbiotic with sea anemones in the Gulf of Aqaba, northern Red Sea. In: Por, F.D., editor. *The Improbable Gulf: History, Biodiversity, and Protection of the Gulf of Aqaba (Eilat)*. Jerusalem: Magnes Press, Hebrew University. 209–223.
- Dixon, A.K., Needham, D., Al-Horani, F.A., Chadwick, N.E. (2014) Microhabitat use and photoacclimation in the clownfish sea anemone *Entacmaea quadricolor*. *Journal of the Marine Biological Association of the United Kingdom* 94(3):473-480.
- Dunn, D.F. (1976) Dynamics of external brooding in the sea anemone *Epictis Epiactis prolifera*. *Marine Biology* 39(1):41-49.
- Edmunds, P.J., Elahi, R. (2007) The demographics of a 15-year decline in cover of the Caribbean reef coral *Montastraea annularis*. *Ecological Monographs* 77(1):3-18.

- Edmunds, P.J. (2010) Population biology of *Porites astreoides* and *Diploria strigosa* on a shallow Caribbean reef. *Marine Ecology Progress Series* 418:87-104.
- Elliott, J.K., Mariscal, R.N. (2001) Coexistence of nine anemonefish species: differential host and habitat utilization, size and recruitment. *Marine Biology* 138:23-36.
- Fautin, D.G., Allen, G.R. (1997) *Anemonefishes and their Host Sea Anemones: A Guide for Aquarists and Divers*. Revised edition. Western Australian Museum, Perth, 160 pp.
- Fong, P., Glynn, P.W. (1998) A dynamic size-structured population model: does disturbance control size structure of a population of the massive coral *Gardineroseris planulata* in the Eastern Pacific? *Marine biology* 130(4):663-674.
- Fricke, H.W. (1983) Social control of sex: field experiments with the anemonefish *Amphiprion bicinctus*. *Zeitschrift Für Tierpsychologie* 61:71-77.
- Glassom, D., Zakai, D., Chadwick-Furman, N.E. (2004) Coral recruitment: a spatio-temporal analysis along the coastline of Eilat, northern Red Sea. *Marine Biology* 144(4):641-651.
- Godwin, J., Fautin D.G. (1992) Defense of host actinians by anemonefishes. *Copeia* 3:902-908.
- Goffredo, S., Mattioli, G., Zaccanti, F. (2004) Growth and population dynamics model of the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). *Coral Reefs* 23:433-443.
- Goffredo, S., Lasker, H.R. (2008) An adaptive management approach to an octocoral fishery based on the Beverton-Holt model. *Coral Reefs* 27:751-761.
- Grigg, R.W. (1984) Resource management of precious corals: a review and application to shallow water reef building corals. *Marine Ecology* 5(1):57-74.
- Gruden, K., Štrukelj, B., Popovič, T., Lenarčič, B., Bevec, T., Brzin, J., et al. (1998) The cysteine protease activity of Colorado potato beetle (*Leptinotarsa decemlineata* Say) guts, which is insensitive to potato protease inhibitors, is inhibited by thyroglobulin type-1 domain inhibitors. *Insect Biochemistry and Molecular Biology* 28(8):549-560.
- Guzner, B., Novoplansky, A., Shalit, O., Chadwick, N.E. (2010) Indirect impacts of recreational scuba diving: patterns of growth and predation in branching stony corals. *Bulletin of Marine Science* 86:727-742.
- Hanski, I. (1999) *Metapopulation ecology*. Oxford University Press. 328 pp.

- Haywood, M., Wells, S. (1989) *The Manual of Marine Invertebrates*. Tetra Press, Salamander Books. Blacksburg, Virginia, USA. 208 pp.
- Hattori, A. (1994) Inter-group movement and mate acquisition tactics of the protandrous anemonefish, *Amphiprion clarkii*, on a Coral Reef, Okinawa. *Japanese Journal of Ichthyology* 41:159-165.
- Hattori, A. (2002) Small and large anemonefishes can coexist using the same patchy resources on a coral reef, before habitat destruction. *Journal of Animal Ecology* 71(5):824-831.
- Hattori, A. (2006) Vertical and horizontal distribution patterns of the giant sea anemone *Heteractis crispa* with symbiotic anemonefish on a fringing coral reef. *Journal of Ethology* 24:51-57.
- Hill, R., Scott, A. (2012) The influence of irradiance on the severity of thermal bleaching in sea anemones that host anemonefish. *Coral Reefs* 31:273-284.
- Hill, R., Fernance, C., Wilkinson, S.P., Davy, S.K., Scott, A. (2014) Symbiont shuffling during thermal bleaching and recovery in the sea anemone *Entacmaea quadricolor*. *Marine Biology* 161(12):2931-2937.
- Hirose, Y. (1985) Habitat, distribution, and abundance of coral reef sea-anemones (Actiniidae and Stichodactylidae) in Sesoko Island, Okinawa, with notes on expansion and contraction behavior. *Galaxea* 4:113-127.
- Hobbs, J.P.A., Frisch, A.J., Ford, B.M., Thums, M., Saenz-Agudelo, P., Furby, K.A., Berumen, M.L. (2013) Taxonomic, Spatial and Temporal Patterns of Bleaching in Anemones Inhabited by Anemonefishes. *PLoS ONE* 8(8):e70966.
- Holbrook, S.J., Schmitt, R.J. (2004) Population dynamics of a damselfish: effects of a competitor that also is an indirect mutualist. *Ecology* 85(4):979-985.
- Holbrook, S.J., Schmitt, R.J. (2005) Growth, reproduction and survival of a tropical sea anemone (Actiniaria): benefits of hosting anemonefish. *Coral Reefs* 24:67-73.
- Huebner, L.K., Chadwick, N.E. (2012) Reef fishes use sea anemones as visual cues for cleaning interactions with shrimp. *Journal of Experimental Marine Biology and Ecology* 416:237-242.
- Huebner, L.K., Dailey, B., Titus, B.M., Khalaf, M., Chadwick, N.E. (2012) Host preference and habitat segregation among Red Sea anemonefish: effects of sea anemone traits and fish life stage. *Marine Ecology Progress Series* 464:1-15.

- Hughes, T.P. (1984) Population dynamics based on individual size rather than age: a general model with a reef coral example. *American Naturalist* 123(6):778-795.
- Hughes, T.P., Jackson, J.B.C. (1985) Population dynamics and life histories of foliaceous corals. *Ecological Monographs* 55:141-166.
- Hughes, T. P., & Tanner, J. E. (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. *Ecology*, 81(8), 2250-2263.
- Hughes, T.P., Baird, A.H., Dinsdale, E.A., Moltschaniwskyj, N.A., Pratchett, M.S., Tanner, J.E., Willis, B.L. (2000) Supply-side ecology works both ways: the link between benthic adults, fecundity, and larval recruits. *Ecology* 81(8):2241-2249.
- Jackson, J.B.C. (1991) Adaptation and diversity of reef corals. *Bioscience* 41:475-482.
- Jarrold, M.D., Calosi, P., Verberk, W.C., Rastrick, S.P., Atfield, A., Spicer, J.I. (2013) Physiological plasticity preserves the metabolic relationship of the intertidal non-calcifying anthozoan-*Symbiodinium* symbiosis under ocean acidification. *Journal of Experimental Marine Biology and Ecology* 449:200-206.
- Jennison, B.L. (1981). Reproduction in three species of sea anemones from Key West, Florida. *Canadian Journal of Zoology* 59(9):1708-1719.
- Jones, A.M., Gardner, S., Sinclair, W. (2008) Losing 'Nemo': bleaching and collection appear to reduce inshore populations of anemonefishes. *Journal of Fish Biology*, 73(3):753-761.
- Lee, K.M., Xie, J.Y., Sun, Y., Kei, K., Qiu, J.W. (2015) Four dense assemblages of the bulb-tentacle sea anemone *Entacmaea quadricolor* and associated clownfish in Hong Kong. *Journal of the Marine Biological Association of the United Kingdom* 95(1):63-68.
- Leslie, P.H. (1945) On the use of matrices in certain population mathematics. *Biometrika* 33:183-212.
- Levitan, D.R. (1991) Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. *The Biological Bulletin* 181(2):261-268.
- Loya, Y. (1972) Community structure and species diversity of hermatypic corals at Eilat, Red Sea. *Marine Biology* 13(2):100-123.
- Loya, Y. (1975). Possible effects of water pollution on the community structure of Red Sea corals. *Marine Biology* 29(2):177-185.

- Loya, Y., Lubinevsky, H., Rosenfeld, M., Kramarsky-Winter, E. (2004) Nutrient enrichment caused by in situ fish farms at Eilat, Red Sea is detrimental to coral reproduction. *Marine Pollution Bulletin* 49(4):344-353.
- Mariscal, R.N. (1972) Behaviour of symbiotic fishes and sea anemones. pp. 327–360. In: Winn, H.E. & Olla, B.L, editors. *Behaviour of Marine Animals, Volume 2*. Plenum Press, New York.
- McCammon, A., Nemeth, D., Sikkel, P. (2007) Anemone-shrimp cleaning stations control ectoparasite loads on Caribbean reef fishes. Annual Meeting of the Gulf and Caribbean Fisheries Institute, Book of Abstracts 60:116.
- Mitchell, J.S. (2003) Mobility of *Stichodactyla gigantea* sea anemones and implications for resident false clown anemonefish, *Amphiprion ocellaris*. *Environmental biology of fishes* 66(1):85-90.
- Nakano, R., Fujii, T. (2014) The soft-coral associated pistol shrimp *Synalpheus neomeris* (De Man)(Decapoda: Alpheidae) defends its host against nudibranchs in Okinawa, Japan. *Raffles Bulletin of Zoology* 62:759-763.
- Nelson, M.W. (2008) Population dynamic modeling of the corkscrew sea anemone *Bartholomea annulata* on Caribbean coral reefs. MSc thesis, Auburn University, Auburn, AL.
- Odum, E.P. (1983) *Basic ecology*. Saunders College Publishing, Philadelphia. 613 pp.
- O'Reilly, E.E. (2015) Demography of the corkscrew sea anemone *Bartholomea annulata* in the Florida Keys and in laboratory culture: A giant sea anemone under pressure. MSc thesis, Auburn University, Auburn, AL.
- Ottoway, J.R. (1978) Population ecology of the intertidal anemone *Actinia tenebrosa*. I. Pedal locomotion and intraspecific aggression. *Australian Journal of Marine & Freshwater Research* 29:787-802.
- Ottoway, J.R. (1979) Population ecology of the intertidal anemone *Actinia tenebrosa*. III. Dynamics and environmental factors. *Australian Journal of Marine & Freshwater Research* 30:41-62.
- Ottoway, J.R. (1980) Population ecology of the intertidal anemone *Actinia tenebrosa*. IV. Growth rates and longevities. *Australian Journal of Marine & Freshwater Research* 31:385-395.
- Outchkourov, N.S., De Kogel, W.J., Wieggers, G.L., Abrahamson, M., Jongasma, M.A. (2004) Engineered multidomain cysteine protease inhibitors yield resistance against western flower thrips (*Frankliniella occidentalis*) in greenhouse trials. *Plant Biotechnology Journal* 2(5):449-458.

- Patton, W.K. (1976) Animal associates of living reef corals. *Biology and geology of coral reefs* 3:1-37.
- Porat, D., Chadwick-Furman, N.E. (2004) Effects of anemonefish on giant sea anemones: expansion behavior, growth, and survival. *Hydrobiologia* 531: 513–520.
- Ramezanpour, M., Burke da Silva, K., Sanderson, B.J. (2012). Differential susceptibilities of human lung, breast and skin cancer cell lines to killing by five sea anemone venoms. *Journal of Venomous Animals and Toxins including Tropical Diseases* 18(2):157-163.
- Ramezanpour, M., Da Silva, K.B., Sanderson, B. J. (2014) Venom present in sea anemone (*Heteractis magnifica*) induces apoptosis in non-small-cell lung cancer A549 cells through activation of mitochondria-mediated pathway. *Biotechnology letters* 36(3):489-495.
- Rhyne, A., Rotjan, R., Bruckner, A., Tlusty, M. (2009) Crawling to collapse: ecologically unsound ornamental invertebrate fisheries. *PLoS One* 4(12):e8413.
- Richardson, D. L., Harriott, V. J., & Harrison, P. L. (1997) Distribution and abundance of giant sea anemones (Actiniaria) in subtropical eastern Australian waters. *Marine and Freshwater Research* 48(1):59-66.
- Richardson, D.L. (1999) Correlates of environmental variables with patterns in the distribution and abundance of two anemonefishes (Pomacentridae: *Amphiprion*) on an eastern Australian sub-tropical reef system. *Environmental Biology of Fishes* 55:255-263.
- Rinkevich, B. (2005) What do we know about Eilat (Red Sea) reef degradation? A critical examination of the published literature. *Journal of Experimental Marine Biology and Ecology* 327(2):183-200.
- Roopin, M., Henry, R.P., Chadwick, N.E. (2008) Nutrient transfer in a marine mutualism: patterns of ammonia excretion by anemonefish and uptake by giant sea anemones. *Marine Biology* 154:547–556.
- Roopin, M., Chadwick, N.E. (2009) Benefits to host sea anemones from ammonia contributions of resident anemonefish. *Journal of Experimental Marine Biology and Ecology* 370:27-34.
- Scott, A., Harrison, P. (2007a) Broadcast spawning of two species of sea anemone, *Entacmaea quadricolor* and *Heteractis crispa*, that host anemonefish. *Invertebrate Reproduction and Development* 50(3):163-171.

- Scott, A., Harrison, P.L. (2007b) Embryonic and larval development of the host sea anemones *Entacmaea quadricolor* and *Heteractis crispa*. *The Biological Bulletin* 213(2):110-121.
- Scott, A., Harrison, P.L. (2008) Larval settlement and juvenile development of sea anemones that provide habitat for anemonefish. *Marine Biology* 154(5):833-839.
- Scott, A., Harrison, P.L. (2009) Gametogenic and reproductive cycles of the sea anemone, *Entacmaea quadricolor*. *Marine Biology* 156(8):1659-1671.
- Scott, A., Malcolm, H.A., Damiano, C., Richardson, D.L. (2011) Long-term increases in abundance of anemonefish and their host sea anemones in an Australian marine protected area. *Marine and Freshwater Research* 62(2):87-196.
- Scott, A., Baird, A.H. (2014) Trying to find Nemo: low abundance of sea anemones and anemonefishes on central and southern mid-shelf reefs in the Great Barrier Reef. *Marine Biodiversity* 45(2):327-331.
- Sebens, K.P. (1981) Reproductive ecology of the intertidal sea anemones *Anthopleura xanthogrammica* (Brandt) and *A. elegantissima* (Brandt): body size, habitat, and sexual reproduction. *Journal of Experimental Marine Biology and Ecology* 54(3):225-250.
- Sheppard, C.R.C., Davy, S.K., Pilling, G.M. (2012) The main reef builders and space occupiers. In: *The Biology of Coral Reefs*. Oxford: Oxford University Press. 33-65.
- Shick, J.M. (1991) *A Functional biology of sea anemones*. Chapman & Hall. 395 pp.
- Shuman, C.S., Hodgson, G., Ambrose, R.F. (2005) Population impacts of collecting sea anemones and anemonefish for the aquarium trade in the Philippines. *Coral Reefs* 24:564-573.
- Suggett, D.J., Hall-Spencer, J.M., Rodolfo-Metalpa, R., Boatman, T.G., Payton, R., Pettay, D.T., Johnson, V.R., Warner, M.E., and Lawson, T. (2012) Sea anemones may thrive in a high CO<sub>2</sub> world. *Global Change Biology* 18(10):3015-3025.
- Swearer, S.E., Shima, J.S., Hellberg, M.E., Thorrold, S.R., Jones, G.P., Robertson, D.R., et al. (2002) Evidence of self-recruitment in demersal marine populations. *Bulletin of Marine Science* 70(Supplement 1):251-271.
- Szczebak, J.T., Henry, R.P., Al-Horani, F.A., Chadwick, N.E. (2013) Anemonefish oxygenate their anemone hosts at night. *Journal of Experimental Biology* 216:970-976.

- Szmant, A.M. (2002) Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? *Estuaries* 25(4):743-766.
- Thornhill, D.J. (2012) Ecological Impacts and Practices of the Coral Reef Wildlife Trade. Defenders of Wildlife, Washington D.C., 179 pp.  
<http://www.defenders.org/sites/default/files/publications/ecological-impacts-and-practices-of-the-coral-reef-wildlife-trade.pdf>.
- Titus, B.M. (2011) Effects of habitat variation on life history traits and genetic structure of *Bartholomea annulata* on Caribbean coral reefs. MSc thesis, Auburn University, Auburn, AL.
- Titus, B.M., Daly, M., Exton, D.A. (2015) Temporal patterns of Pederson shrimp (*Ancylomenes pedersoni* Chace 1958) cleaning interactions on Caribbean coral reefs. *Marine Biology* 162(8):1651-1664.
- Upadhyay, S.K., Eckel-Mahan, K.L., Mirbolooki, M.R., Tjong, I., Griffey, S.M., Schmunk, G., et al. (2013) Selective Kv1.3 channel blocker as therapeutic for obesity and insulin resistance. *Proceedings of the National Academy of Sciences* 110(24):E2239-E2248.
- Valizadeh, M., Deraison, C., Kazemitabar, S.K., Rahbé, Y., Jongsma, M.A. (2013) Aphid Resistance in Florist's Chrysanthemum (*Chrysanthemum morifolium* Ramat.) Induced by Sea Anemone Equistatin Overexpression. *African Journal of Biotechnology, Academic Journals* 12(50):6922-6930.
- Wabnitz, C., Taylor, M., Green, E., Razak, T., et al. (2003) From ocean to aquarium: the global trade in marine ornamental species. UNEP-WCMC, Cambridge.
- Wedi, S.E., Dunn, D.F. (1983) Gametogenesis and reproductive periodicity of the subtidal sea anemone *Urticina lofotensis* (Coelenterata: Actiniaria) in California. *The Biological Bulletin* 165(2):458-472.
- Wielgus, J., Glassom, D., Fishelson, L. (2003) Long-term persistence of low coral cover and abundance on a disturbed coral reef flat in the northern Red Sea. *Journal of Experimental Marine Biology and Ecology* 297(1):31-41.
- Wilkinson, C.R. (Ed.) (2008) Status of coral reefs of the world. Global Coral Reef Monitoring Network. <http://gcrmn.org/gcrmn-publication/status-of-coral-reefs-of-the-world-2008>.
- Wilson, S.K., Graham, N.A.J., Pratchett, M.S., Jones, G.P., Polunin, N.V.C. (2006) Multiple disturbances and the global degradation of coral reefs: Are reef fishes at risk or resilient? *Global Change Biology* 12:2220-2234.

Wu, B., McVay, M.J., Billor, N., Chadwick, N.E. (2015) Environmental predictors of long-term growth and shrinkage in the clownfish sea anemones *Entacmaea quadricolor* and *Heteractis crispa*. Manuscript in preparation.

Zakai, D., Chadwick-Furman, N.E. (2002) Impacts of intensive recreational diving on reef corals at Eilat, northern Red Sea. *Biological Conservation* 105(2): 179-187.

**Table 2.1.** Annual transition matrices over 4 years for 2 species of sea anemones on coral reefs at Eilat, Israel. Columns refer to the proportion of individuals in each size class: I (< 150 cm<sup>2</sup> tentacle crown surface area, TCSA), II (150-300 cm<sup>2</sup> TCSA), and III (> 300 cm<sup>2</sup> TCSA), that transitioned to other size classes (rows) during the transition period, or that died ( $q_x$  = probability of mortality,  $q_x N$  = number of individuals that died) or recruited into the size class ( $R_n$  = number of recruits). N = total number of individuals per size class at the beginning of each period. Bold cells represent parameters with highest elasticity for each year.

	1996-1997			1997-1998			1998-1999			1999-2000		
	I	II	III	I	II	III	I	II	III	I	II	III
<b>I</b>	0.38	0.12	0.10	<b>0.56</b>	0.37	0.24	<b>0.67</b>	0.52	0.31	0.36	0.28	0.11
<b>II</b>	0.18	0.17	0.11	0.06	0.32	0.40	0.13	0.26	0.31	0.11	0.39	0.11
<b>III</b>	0.27	0.67	<b>0.74</b>	0.03	0.05	0.26	0.00	0.15	0.38	0.15	0.28	<b>0.56</b>
$q_x$	0.18	0.04	0.05	0.35	0.26	0.10	0.20	0.07	0.00	0.38	0.05	0.22
$q_x N$	8	1	1	11	5	4	8	2	0	18	1	2
$R_n$	10	5	--	5	2	--	2	2	--	13	0	--
N	45	24	19	32	19	42	40	27	13	47	18	9

	1996-1997			1997-1998			1998-1999			1999-2000		
	I	II	III	I	II	III	I	II	III	I	II	III
<b>I</b>	0.56	0.05	0.15	<b>0.54</b>	0.23	0.19	<b>0.59</b>	0.32	0.31	0.54	0.16	0.00
<b>II</b>	0.23	0.37	0.09	0.20	0.35	0.16	0.09	0.26	0.41	0.20	0.40	0.11
<b>III</b>	0.00	0.36	<b>0.67</b>	0.02	0.23	<b>0.57</b>	0.00	0.07	0.24	0.12	0.08	<b>0.78</b>
$q_x$	0.21	0.22	0.09	0.24	0.19	0.08	0.32	0.35	0.04	0.14	0.36	0.11
$q_x N$	7	9	3	10	6	3	15	11	1	8	9	1
$R_n$	15	5	--	10	6	--	10	1	--	14	6	--
N	34	41	33	41	31	37	46	31	29	56	25	9

**B.** Leathery sea anemones *Heteractis crispa*

**Table 2.2.** Supplemental annual transition matrices with recruitment rates summed into corresponding vital rates for 2 species of sea anemones on coral reefs at Eilat, Israel. Columns refer to the proportion of individuals in each size class: I ( $< 150 \text{ cm}^2$  tentacle crown surface area, TCSA), II ( $150\text{-}300 \text{ cm}^2$  TCSA), and III ( $> 300 \text{ cm}^2$  TCSA), that transitioned to other size classes (rows) during the transition period, or that died ( $q_x$  = probability of mortality,  $q_x N$  = number of individuals that died) or recruited into the size class ( $R_n$  = number of recruits).  $N$  = total number of individuals per size class at the beginning of each period. Bold cells represent parameters with highest elasticity for each year.

	1996-1997			1997-1998			1998-1999			1999-2000		
	I	II	III	I	II	III	I	II	III	I	II	III
	<b>I</b>	0.40	0.25	0.42	<b>0.58</b>	0.45	0.31	<b>0.68</b>	0.54	0.40	0.39	0.50
<b>II</b>	0.19	0.23	0.26	0.07	0.35	0.43	0.13	0.28	0.40	0.11	0.39	0.11
<b>III</b>	0.27	0.67	<b>0.74</b>	0.03	0.05	0.26	0.00	0.15	0.38	0.15	0.28	<b>0.56</b>
$q_x$	0.18	0.04	0.05	0.35	0.26	0.10	0.20	0.07	0.00	0.38	0.05	0.22
$q_x N$	8	1	1	11	5	4	8	2	0	18	1	2
$R_n$	10	5	--	5	2	--	2	2	--	13	0	--
$N$	45	24	19	32	19	42	40	27	13	47	18	9

**D. Leathery sea anemones *Heteractis crispa***

	1996-1997			1997-1998			1998-1999			1999-2000		
	I	II	III	I	II	III	I	II	III	I	II	III
	<b>I</b>	0.60	0.16	0.42	<b>0.56</b>	0.32	0.35	<b>0.61</b>	0.42	0.52	0.56	0.33
<b>II</b>	0.25	0.40	0.18	0.21	0.41	0.26	0.09	0.27	0.43	0.21	0.47	0.51
<b>III</b>	0.00	0.37	<b>0.67</b>	0.02	0.23	<b>0.57</b>	0.00	0.06	0.24	0.13	0.08	<b>0.78</b>
$q_x$	0.21	0.22	0.09	0.24	0.19	0.08	0.32	0.35	0.04	0.14	0.36	0.11
$q_x N$	7	9	3	10	6	3	15	11	1	8	9	1
$R_n$	15	5	--	10	6	--	10	1	--	14	6	--
$N$	34	41	33	41	31	37	46	31	29	56	25	9

**Table 2.3.** Elasticity values for *Entacmaea quadricolor* transition matrices over 4 years at Eilat, Israel without recruitment rates added into the matrix elements (A) and with recruitment rates added in (B).

	1996-1997			1997-1998			1998-1999			1999-2000		
	I	II	III									
I	0.056	0.015	0.067	0.536	0.098	0.024	0.546	0.110	0.022	0.095	0.058	0.050
II	0.033	0.026	0.082	0.072	0.105	0.049	0.132	0.072	0.028	0.052	0.144	0.090
III	0.049	0.100	0.574	0.050	0.023	0.044	0.000	0.050	0.041	0.057	0.084	0.370
N	45	24	19	32	19	42	40	27	13	47	18	9

**A. Without recruitment added in**

	1996-1997			1997-1998			1998-1999			1999-2000		
	I	II	III									
I	0.072	0.027	0.113	0.495	0.108	0.025	0.496	0.111	0.025	0.134	0.046	0.169
II	0.052	0.039	0.109	0.082	0.123	0.050	0.137	0.084	0.037	0.076	0.072	0.038
III	0.088	0.134	0.365	0.051	0.024	0.041	0.000	0.062	0.048	0.138	0.069	0.258
N	45	24	19	32	19	42	40	27	13	47	18	9

**B. With recruitment added in**

**Table 2.4.** Elasticity values for *Heteractis crispa* transition matrices over 4 years at Eilat, Israel without recruitment rates added into the matrix elements (A) and with recruitment rates added in (B).

**A. Without recruitment added in**

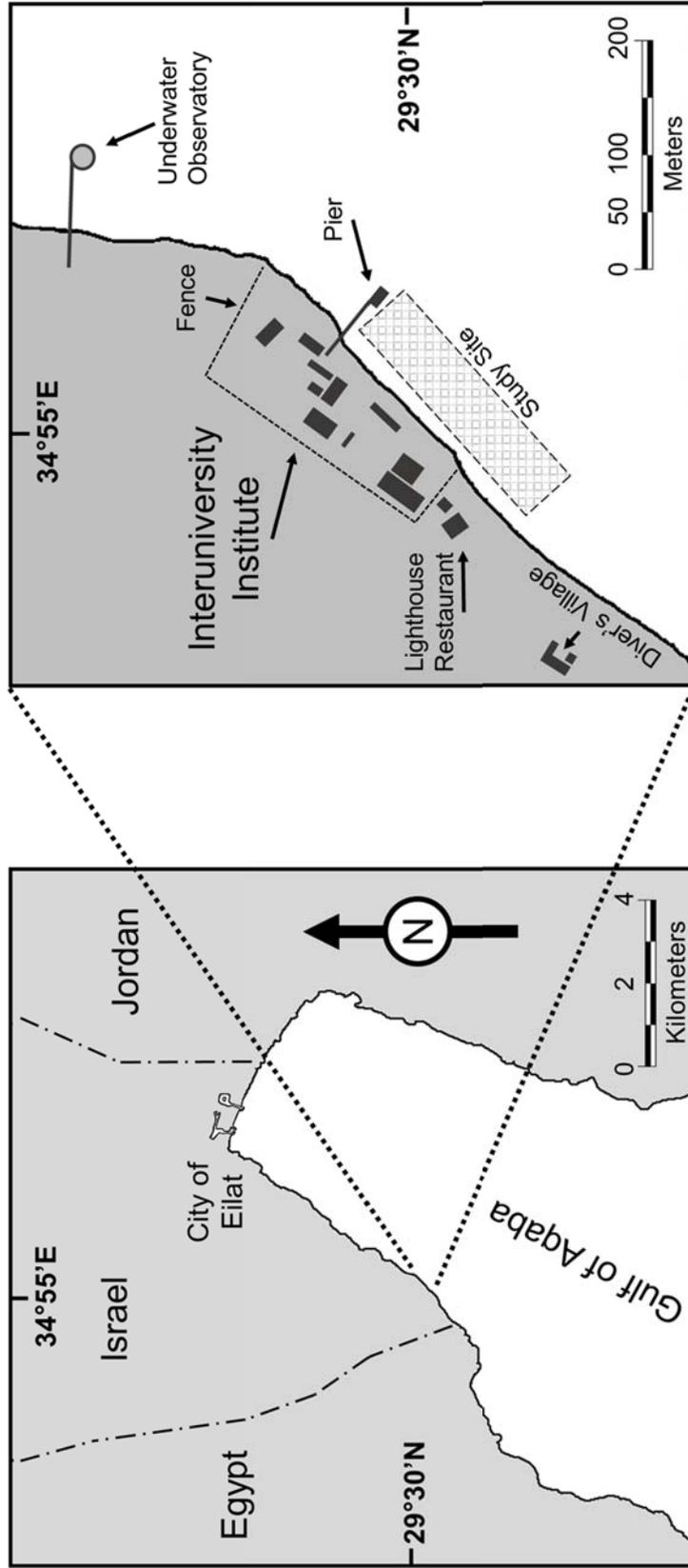
	1996-1997			1997-1998			1998-1999			1999-2000		
	I	II	III									
<b>I</b>	0.149	0.010	0.066	0.228	0.064	0.055	0.660	0.089	0.013	0.069	0.037	0.000
<b>II</b>	0.076	0.095	0.049	0.106	0.121	0.058	0.102	0.073	0.017	0.016	0.058	0.047
<b>III</b>	0.000	0.115	0.439	0.013	0.099	0.256	0.000	0.030	0.016	0.021	0.026	0.725
<b>N</b>	34	41	33	41	31	37	46	31	29	56	25	9

**B. With recruitment added in**

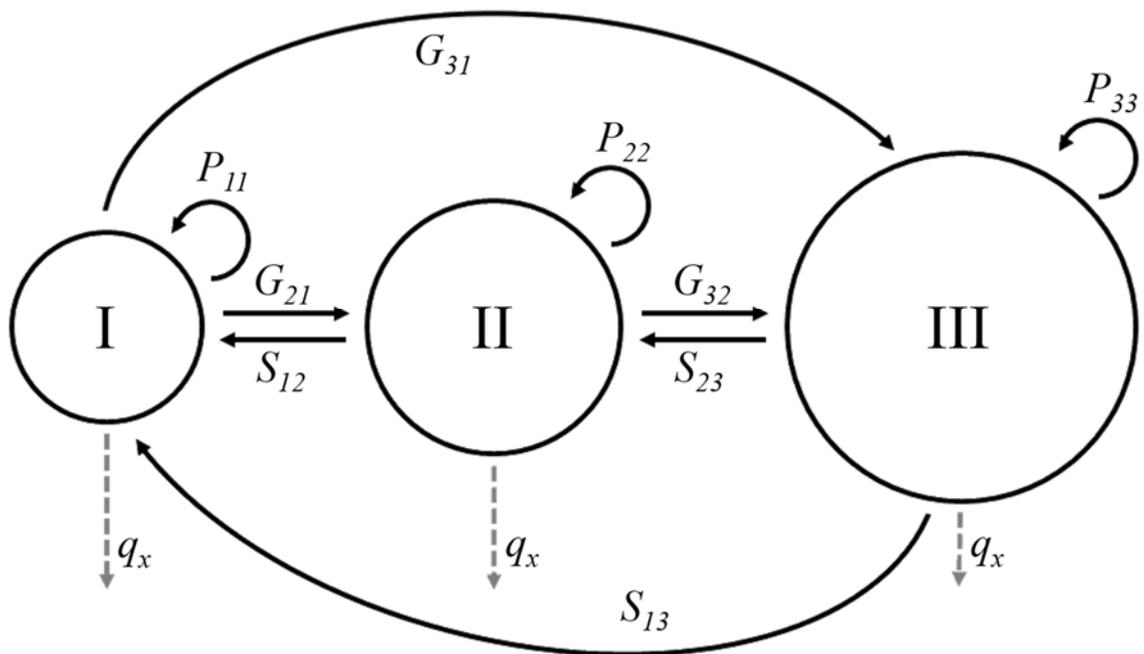
	1996-1997			1997-1998			1998-1999			1999-2000		
	I	II	III									
<b>I</b>	0.172	0.028	0.084	0.208	0.077	0.059	0.625	0.099	0.016	0.158	0.056	0.113
<b>II</b>	0.112	0.115	0.058	0.121	0.146	0.066	0.115	0.078	0.017	0.051	0.071	0.054
<b>III</b>	0.000	0.142	0.290	0.015	0.110	0.198	0.000	0.033	0.016	0.118	0.048	0.331
<b>N</b>	34	41	33	41	31	37	46	31	29	56	25	9

**Table 2.5.** Variation in rates of population turnover time ( $T$ , years) among years and between species of giant sea anemones *Entacmaea quadricolor* and *Heteractis crispa*, on coral reefs at Eilat, Israel, northern Red Sea. Numbers indicate the number of individuals at the study site in each event category for each period. Population turnover time ( $T$ ) was calculated as:  $[(M + R) / (N_t + N_{t+1})]^{-1}$  where  $M$  = number of individuals that died (lost from the population),  $R$  = number of recruits,  $N_t$  = number of individuals at the beginning of the annual period, and  $N_{t+1}$  = number that survived to the end of the annual period.

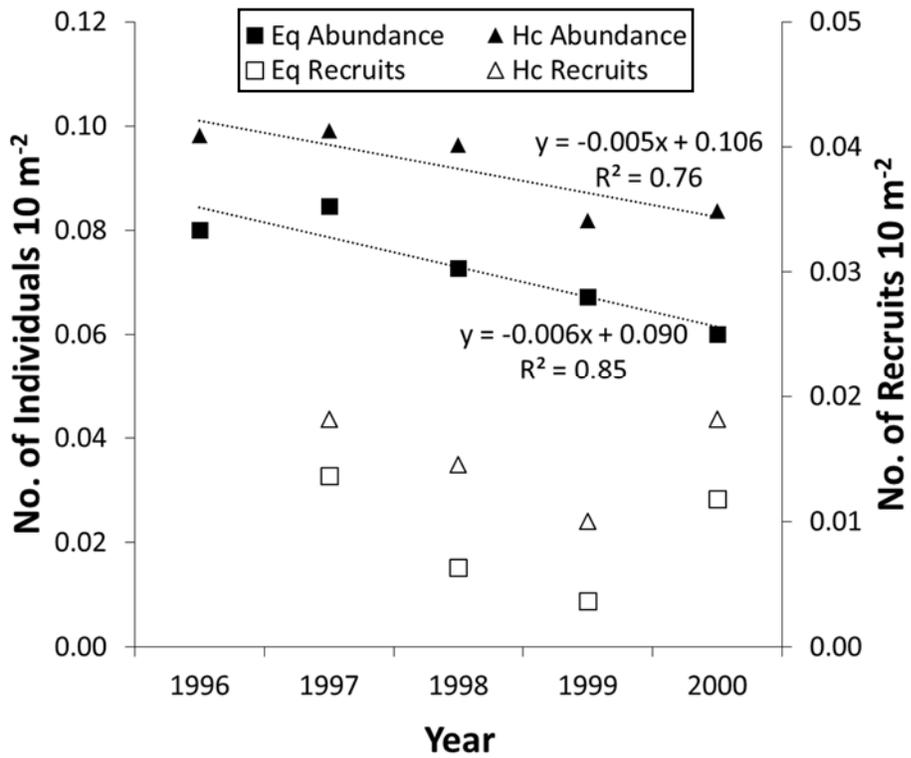
Species and Year	Event				$T =$
	Start	Lost	Recruited	Remain	
<i>E. quadricolor</i>					
1996	88	10	15	93	7.24
1997	93	20	7	80	6.41
1998	80	10	4	74	11.00
1999	74	21	13	66	4.12
$T = 7.19 \pm 2.48$ years ( $\bar{x} \pm SD$ )					
<i>H. crispa</i>					
1996	108	19	20	109	5.56
1997	109	19	16	106	6.14
1998	106	27	11	90	5.16
1999	90	18	20	92	4.79
$T = 5.41 \pm 0.50$ years ( $\bar{x} \pm SD$ )					



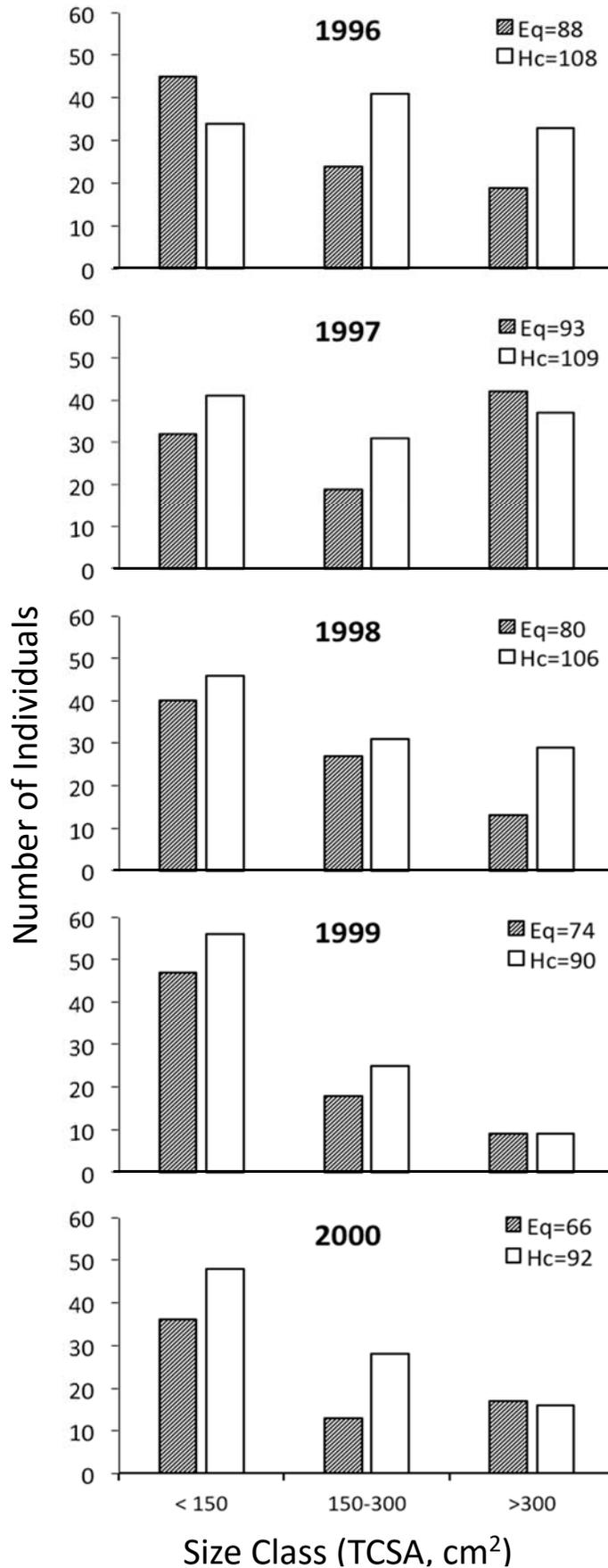
**Figure 2.1.** Map of the northern Gulf of Aqaba, Red Sea, showing location of study site near Eilat, Israel, and detail of study site adjacent to the Interuniversity Institute in Eilat.



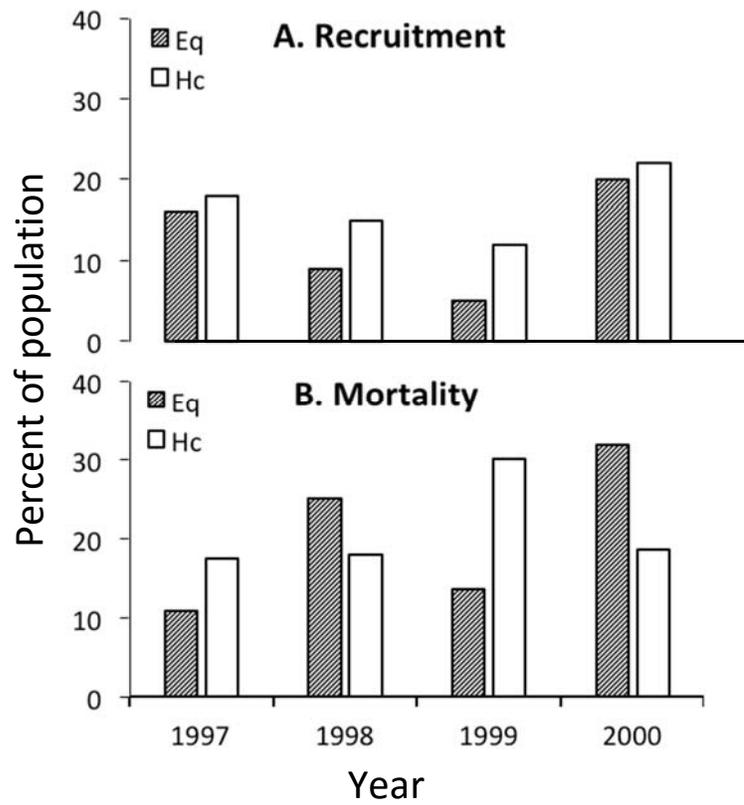
**Figure 2.2.** Schematic illustration of the 3 sea anemone size classes examined here, and the parameters that control their size-frequency distributions.  $G$  = growth,  $S$  = shrinkage,  $P$  = stasis (retention in the same size class), and  $q_x$  = mortality. Rates refer to the probabilities for transition of individuals as a result of transitions between or into/out of each size class, where the subscript numbers designate the destination size class ( $i$ , first digit), and the size class of origin ( $j$ , second digit).



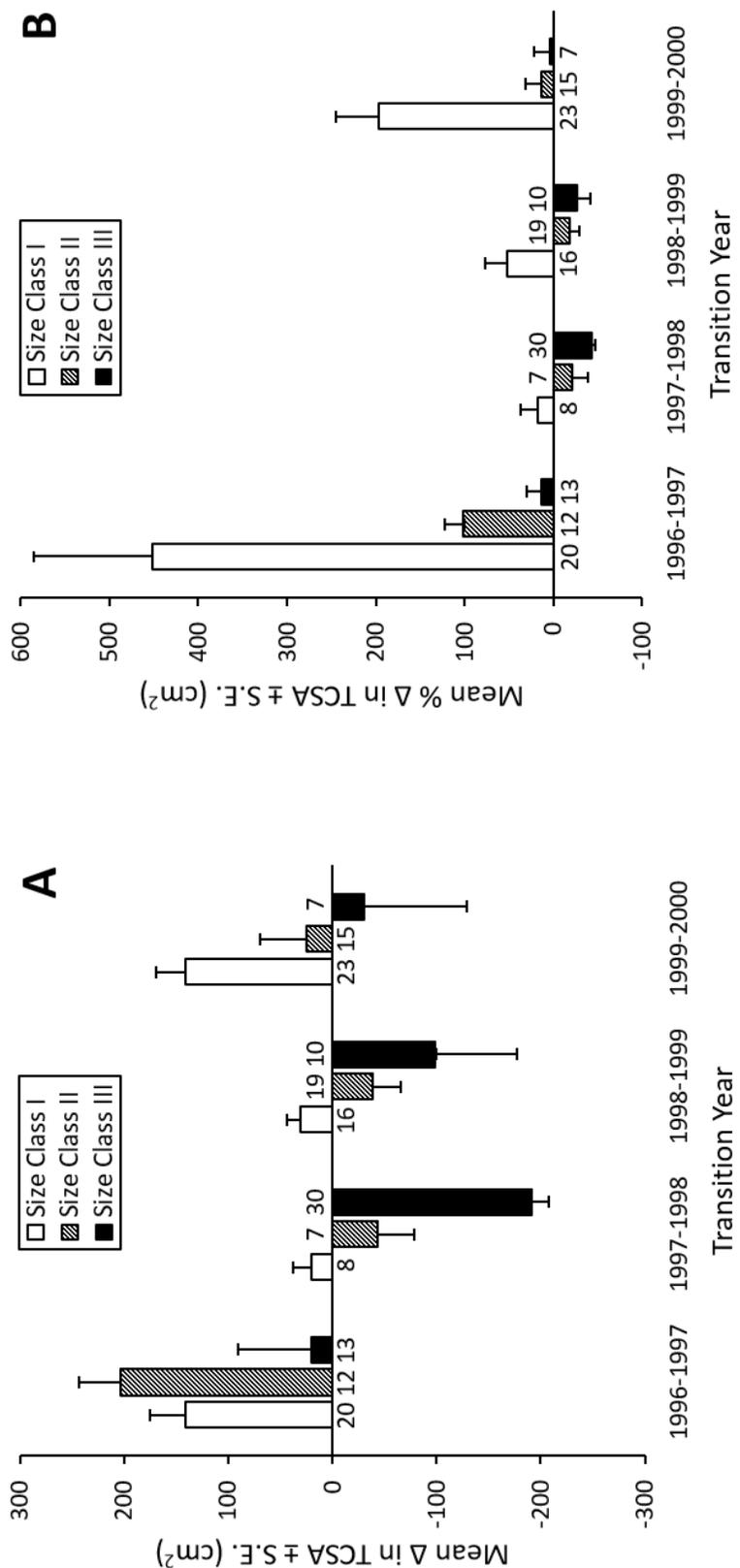
**Figure 2.3.** Variation over 5 years in the total abundance of individuals of bulb-tentacle sea anemones *Entacmaea quadricolor* (Eq) and leathery anemones *Heteractis crispa* (Hc), and in the abundance of recruits of both species, on coral reefs at Eilat, Israel, northern Red Sea.



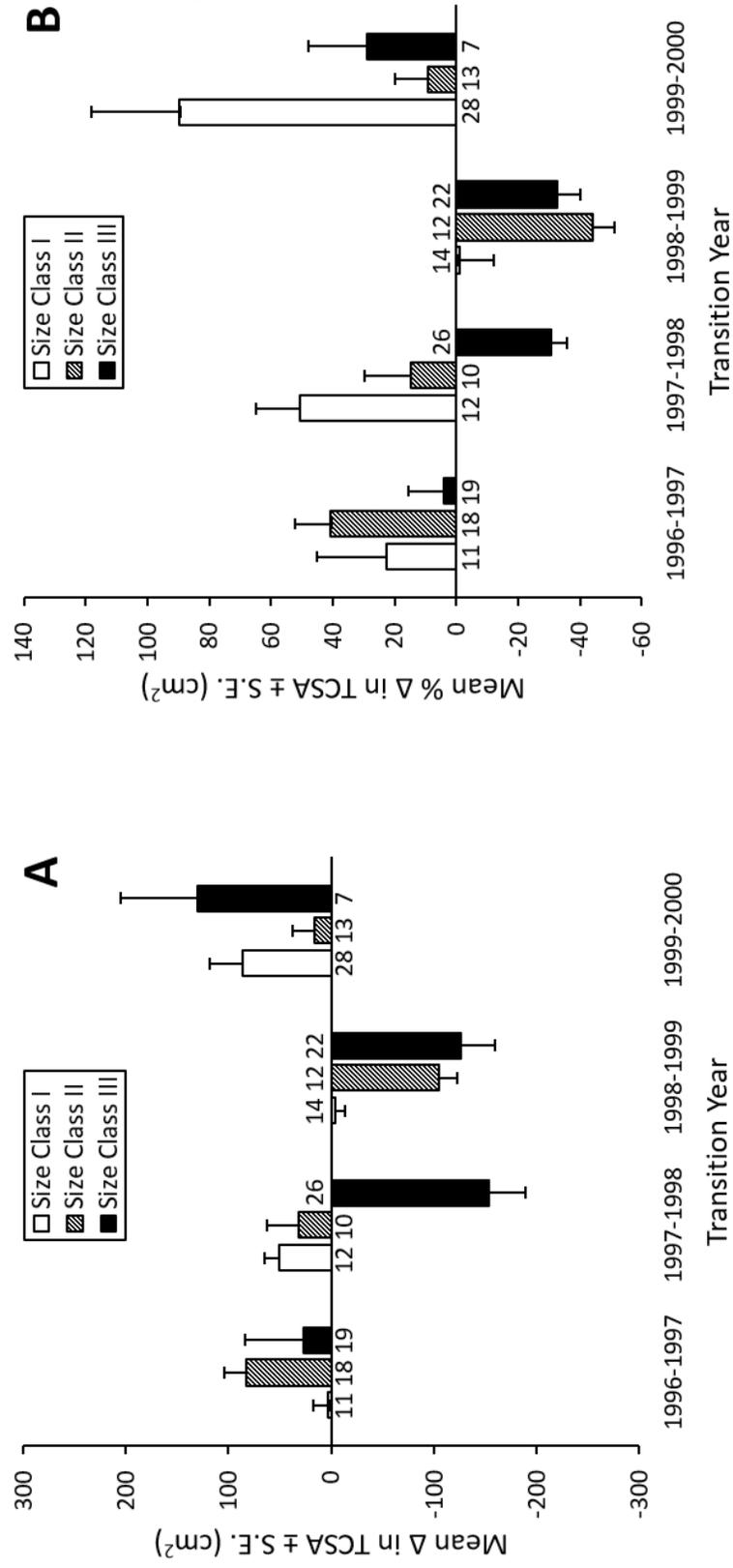
**Figure 2.4.** Variation in population size structure among years and species, for bulb-tentacle sea anemones *Entacmaea quadricolor* (Eq) and leathery sea anemones *Heteractis crispa* (Hc), on coral reefs at Eilat, Israel, northern Red Sea. Abundance (number of individuals) was determined within a 220 x 50 m study site (see site map, Fig. 2.1); TCSA = tentacle crown surface area (cm<sup>2</sup>); total population size for each species is shown in bar legends.



**Figure 2.5.** Variation in rates of recruitment (A) versus mortality (B) among years and between species of giant sea anemones *Entacmaea quadricolor* (Eq) and *Heteractis crispa* (Hc) on coral reefs at Eilat, Israel, northern Red Sea.



**Figure 2.6.** Variation in patterns of change in body size among years and size classes in the bulb-tentacle sea anemone *Entacmaea quadricolor* in terms of absolute change (A) and percent change (B) in tentacle crown surface area (TCSA) (mean  $\pm$  S.E.) at Eilat, Israel, 1996-2000. Size classes are: I = < 150 cm<sup>2</sup>, II = 150-300 cm<sup>2</sup>, III = > 300 cm<sup>2</sup>. Numbers at the base of each column represent the sample size in each size class at each period.



**Figure 2.7.** Variation in patterns of change in body size among years and size classes in the leathery sea anemone *Heteractis crispata* in terms of absolute change (A) and percent change (B) in tentacle crown surface area (TCSA) (mean  $\pm$  S.E.) at Eilat, Israel, 1996-2000. Size classes are: I = < 150 cm<sup>2</sup>, II = 150-300 cm<sup>2</sup>, III = > 300 cm<sup>2</sup>. Numbers at the base of each column represent the sample size in each size class at each period.

## Chapter 3

### Long-term decline of clownfish sea anemones in the Israeli Red Sea and effects on obligate anemonefish associates

#### Abstract

Giant sea anemones provide important habitat and protection for diverse symbiotic associates, including microalgae, shrimps, and fishes on Indo-Pacific coral reefs. Anthropogenic factors, especially climate change, nutrient pollution, and overfishing, are negatively impacting coral reefs in many regions and potentially causing populations of these anemones to decline, yet almost nothing is known about long-term demographic trends in giant coral reef sea anemones and their obligate anemonefish residents. We examined populations of Red Sea-endemic two-band anemonefish *Amphiprion bicinctus* and their 2 main hosts in the northern Red Sea, bulb-tentacle sea anemones *Entacmaea quadricolor* and leathery sea anemones *Heteractis crispa*, from 1996-2014 on coral reefs at Eilat, Israel. Demographic processes revealed during the initial intensive, annual sampling during 1996-2000 were compared to a follow-up study during 2013-14. Populations of both anemone species continued to decline as predicted, reaching all-time lows in their abundances in 2014. As the anemonefish population became progressively space-limited due to lack of host habitat area, the mean body size of the fish increased, likely due to larger, more dominant individuals preventing smaller immigrants (both larval recruits from the plankton and fish migrating from other anemones) from

inhabiting the increasingly-limited anemone space. The proportion of anemones that hosted adult breeding pairs of anemonefish tripled between 1996 and 2014, but the total number of anemonefish breeding pairs remained relatively unchanged. Leathery anemones *H. crispera* continued to decline in 2013-2014 at the same rate as observed previously during 1996-2000. In contrast, bulb-tentacle anemones *E. quadricolor* declined less precipitously, probably because they were the preferred host into which ever-larger anemonefish crowded, thus contributing mutualistic benefits that slowed host population loss. I conclude that anemonefish appear to slow the long-term decline of *E. quadricolor*, but not *H. crispera*. Conservation measures are urgently needed to prevent the local extinction of these important mutualists from Israeli coral reefs.

## Introduction

Clownfish sea anemones are ecologically and commercially valuable coral reef organisms that are vulnerable to population declines caused by anthropogenic stressors such as overfishing (Shuman et al. 2005) and temperature-induced bleaching (Hill and Scott 2012, Hobbs et al. 2013). Information on their long-term population dynamics is needed to design effective management strategies and to provide information about the volume of exploitation that may be sustainable (Scott et al. 2011, Scott and Baird 2014). In particular, researchers need to know how robust population projections are, and how well they predict the ability of populations to recover from overharvesting or other impacts, as well as how quickly they are expected to decline when stressed.

Bulb-tentacle sea anemones *Entacmaea quadricolor* and leathery sea anemones *Heteractis crispa* are obligate hosts for endemic two-band anemonefish *Amphiprion bicinctus* in the Red Sea (Fautin and Allen 1997, Chadwick and Arvedlund 2005, Huebner et al. 2012), and also are the most widespread hosts of the majority of other known species of anemonefishes (20 out of 30) throughout the tropical Indo-Pacific region (Fautin and Allen 1997, Allen et al. 2008, 2010). Chapter 2 of this thesis describes the first detailed demographic analysis of these 2 important sea anemone species, focused on 4 years of data collected ~15-20 years ago. Updated information on these 2 populations is needed, to assess the validity of long-term population projections made for both species, and to understand the biological mechanisms causing deviations from their projected abundances on coral reefs in the Israeli Red Sea.

To address this need, as well as to provide a more complete demographic analysis and robust harvest recommendations, my study site was revisited during 2013-2014 to re-census individuals of both species, thus acquiring a 18-year data set (1996-2014) for the analysis of long-term population trends. This combination of historical and recent data from the same reef area allows the elucidation of long-term population trends and a strong scientific basis for management recommendations for both species, based on their trends of change in body size, mortality, and recruitment over 2 decades.

The initial 4-year population study (Chapter 2) revealed that both species were declining in abundance at substantial linear rates that, if they continued, were expected to lead to the local extinction of *E. quadricolor* and *H. crispa* in 2010 and 2018, respectively. Here, I present evidence that potentially explains why *E. quadricolor* anemones have not yet become locally extinct, and also report on the population

structure, abundance, and anemonefish associates of both host anemone species in 2013 and 2014. I use this information to evaluate the long-term effects of trends initially observed in 1996-2000, and to explore the contrasting impacts of patterns in their symbiosis with anemonefish, which differ substantially between these 2 major species of clownfish sea anemones (Huebner et al. 2012).

## **Methods**

### ***Study site and collection of field data***

The present study was conducted at the northern tip of the Gulf of Aqaba, northern Red Sea, in Eilat, Israel, adjacent to the Interuniversity Institute (IUI) for Marine Science. The study site was a 220 x 50 m area of reef slope that was examined annually during 1996-2000 for all individuals of bulb-tentacle sea anemones *E. quadricolor* and leathery sea anemones *H. crista*, which were marked and monitored for annual assessment of population dynamics (Chapter 2).

During December 2013 and October 2014, the site was revisited, and all individuals of the above 2 species were re-censused using similar methods to those described in Chapter 2. For each individual, the following data were collected: species, tag number, tentacle crown length and width (for calculation of tentacle crown surface area [TCSA], see Chapter 2), depth, substrate type (rock or sand), orientation (vertical or horizontal), number and size (total length, TL) of all two-band anemonefish *A. bicinctus*,

and individual markings to aid in re-identification (tentacle morphology after Huebner et al. [2012], striations on the oral disk or tentacles, etc.).

In contrast to the intensive 1996-2000 study, the anemones examined during 2013-2014 were not marked individually. The mean population turnover times for these 2 species at this site were estimated to be only 5-7 years (Chapter 2), so it is likely that only a few, if any, of the individuals observed in 1996-2000 still remained in the populations in 2013-2014. Additionally, the metal wires that had once attached engraved aluminum identification markers to the reef substrate adjacent to each anemone had corroded during the 13-year interim, and the markers had detached and were lost in the sediment or swept away by currents, and could not be relocated. Therefore, comparison of individual anemone identity with the previous study was not possible due to high population turnover and lost markers for any anemones that may have remained in the population since the previous survey.

During the follow-up surveys, each anemone was temporarily marked by tying a short strand (25 to 30 cm) of fluorescent plastic flagging tape to the hard substrate adjacent to the anemone after it was censused, to ensure that no anemone was censused more than once each year.

Anemonefish were designated as reproductive adults at 6cm TL (after Fricke 1983, Huebner et al. 2012), and 2 adults observed within the same anemone host were considered to be a breeding pair capable of reproduction. Individual *H. crista* anemones were observed only very rarely to host breeding pairs of anemonefish during 1996-2000 (~1% of 505 observations across 5 years of census data), whereas many individuals of *E. quadricolor*, which are preferred hosts for adult *A. bicinctus* (Huebner et al. 2012),

contained breeding fish pairs (~14% of 489 observations across 5 years). Thus, only adult fish pairs associated with the preferred host anemone *E. quadricolor* were considered as contributing to the population of anemonefish breeding pairs in the present analysis.

### ***Data analysis***

The sea anemones were organized by size class (Chapter 2) based on TCSA: I (< 150 cm<sup>2</sup>), II (150-300 cm<sup>2</sup>), or III (> 300 cm<sup>2</sup>), for analysis of population structure. Regression trendlines of change in sea anemone abundance over time during 1996-2000 (Chapter 2) were extended to the date at which they intersected the x-axis ( $y = 0$  individuals left in the populations), to predict when each population was expected to disappear at the study site if the 1996-2000 trends of population decline continued. Then, actual population abundance data from the 2013-2014 censuses were used to create new trendlines of actual change over 18 years, and these were compared with the original trendlines of predicted change (*t*-tests for differences between slopes).

The anemones of each species also were placed into 3 categories based on their containing no fish, only juveniles, or at least 1 adult fish during 2013-2014. The proportion of anemones that hosted each type of anemonefish category was compared between the 2 host species for both years using chi-squared tests. The anemonefish category (juvenile or adult) associated with each host in both years also was compared using chi-squared tests. All results are reported as means  $\pm$  one standard deviation unless indicated otherwise.

## Results

The abundance of both species of host anemones at this Red Sea study site declined precipitously over 18 years, from 0.080 individuals per 10 m<sup>2</sup> in 1996, to only 0.033 individuals per 10 m<sup>2</sup> by 2014 for *E. quadricolor* (Table 3.1A), and from 0.098 to only 0.016 individuals per 10 m<sup>2</sup> for *H. crispa* (Table 3.1B), representing a substantial decrease in population size over almost 2 decades. Even between the 2 years of the recent surveys in 2013 and 2014, the number of anemones at the study site declined from 52 *E. quadricolor* and 37 *H. crispa* in 2013, to only 36 *E. quadricolor* and 18 *H. crispa*, a loss of 1/3 to 1/2 of individuals in only 1 recent year.

Population size structure for both species was skewed from that expected in a stable anemone population, of an inverse relationship between the number of individuals and size class (i.e., many small and few large individuals in each population, as calculated based on the structure of stable populations of these species at a nearby site in Jordan, N.E. Chadwick and M.J. McVay, unpublished data). Instead, the middle size class contained the highest proportion of individuals in both species in 2013, but only for *H. crispa* in 2014 (Fig. 3.1), indicating a marked lack of small individuals in either population during both years. The only size class that retained similar numbers in both species between 2013 and 2014 was the class containing the largest individuals, Size Class III (Fig. 3.1).

The changes in abundance of both anemones that initially were observed during 1996-2000 (see Chapter 2), and which indicated a gradual decline of both populations, in general continued as predicted based on the recent surveys (Fig. 3.2). The predicted

trendline was highly accurate for *H. crispa*, and did not differ much from the actual trendline (Fig. 3.2B). In contrast, the predicted trendline of population loss in *E. quadricolor* had a steeper slope than the actual observed trendline based on the 2013-14 surveys (Fig. 3.2A). The actual trendlines based on all surveys from 1996-2014 indicated that abundance of *E. quadricolor* at this site was expected to reach 0 by 2031 (20 years later than the local extinction predicted for the year 2010 from only the 1996-2000 data, Fig. 3.2A). Conversely, the predicted year of local extinction for *H. crispa* was almost the same based on both the initial (1996-2000) and long-term (1996-2014) datasets, at 2018 and 2019, respectively (Fig. 3.2B).

During the recent surveys, most of the anemones contacted rock or dead coral with their tentacles and pedal discs, rather than sand. The proportion of individuals of both species that attached to rock or dead coral was 93%, with the other 7% attached to sandy substrate. Only leathery anemones were regularly surrounded by soft substrate, with 15-17% of individuals, compared to only 0-1% of *E. quadricolor* individuals.

In terms of anemone body sizes, the 2 species showed contrasting trends of change over 18 years. Compared to 1996, the mean size of *E. quadricolor* anemones was 33-37% larger during recent years (Table 3.1A), but that of *H. crispa* was 13-17% smaller (Table 3.1B). The mean body size within each size class by year showed no significant change during 2013-2014 for either *E. quadricolor* (Table 3.2A) or *H. crispa* (Table 3.2B), but there was a noticeable drop in Size Class III, which represents the largest anemones that contribute the most to sexual reproduction (Chapter 2). During 2013-2014, the mean size of anemones in Size Class III dropped 27% for *E. quadricolor* and 19% for *H. crispa*.

A total of 115 anemonefish were observed at the study site in 2013, but only 68 were present in 2014 (not surprisingly because these fish cannot live without the anemone hosts, numbers during both recent years were much lower than in 1996-2000, when 167 to 265 anemonefish occurred at the site). Anemonefish abundance decreased from 73 to 51 fish hosted by *E. quadricolor*, and 42 to 17 fish hosted by *H. crispa* during 2013-2014. This decrease caps a precipitous decline in anemonefish abundance at the study site over 18 years, in which the number of anemonefish hosted by *E. quadricolor* and *H. crispa* declined by 59% and 88%, respectively. The mean number of anemonefish per host anemone individual remained similar over the 18-year period for *E. quadricolor* (Table 3.1A, range =  $1.22 \pm 0.77$  to  $1.45 \pm 0.61$  fish/anemone), but fluctuated more for *H. crispa* (Table 3.1B, range =  $0.83 \pm 0.73$  to  $1.32 \pm 1.06$  fish/anemone).

Mean total length (TL) of the anemonefish hosted by each anemone species increased since the first observations in 1996, in which TL of fish hosted by *E. quadricolor* increased 37% and those hosted by *H. crispa* increased 23%. The mean sum of the 2 largest fish hosted by *E. quadricolor* increased 39% over 18 years (Table 3.1A), while those hosted by *H. crispa* fluctuated more, rising during some years before dropping back down near their original numbers (Table 3.1B).

In 2013, 57 adult and 51 juvenile anemonefish were observed among the 2 host anemones. Those figures decreased to 37 adults and 29 juveniles in 2014. A significant difference ( $p = < 0.001$ ) occurred between the 2 host species in the level of anemonefish hosted (e.g., juveniles, adults, or no fish) during both years, with *H. crispa* more commonly hosting juveniles or no anemonefish than *E. quadricolor* (Table 3.3). Variation in the total number of adult versus juvenile fish that appeared in the 2 host

species was also observed, with *E. quadricolor* hosting a significantly higher proportion of adult anemonefish (65-72%) than *H. crispa* (6-30%) in both years ( $p < 0.001$ , Table 3.4).

Breeding pairs of anemonefish associated only with *E. quadricolor* in 2013 and 2014. Pairs of reproductive adults occurred in 12 anemones in 2013, and 11 anemones in 2014. These anemone hosts represent 23% and 31% of the *E. quadricolor* individuals in 2013 and 2014, respectively, which continues a long-term upward trend in the proportion of the anemone population that hosted breeding pairs of anemonefish (Table 3.5).

## Discussion

I demonstrate here that the decline in clownfish sea anemones observed during the initial surveys at Eilat in 1996-2000 (Chapter 2) has continued approximately as predicted into the last few years. My analysis indicates that the long-term loss of giant sea anemones at this site likely will continue, and if not mitigated, result in their local extinction, as well as that of the organisms that depend directly or indirectly upon them as obligate symbiotic hosts (both anemonefishes and anemoneshrimps, Chadwick et al. 2008). This report provides a valuable update to the intensive studies carried on at this site in the late 1990s, resulting in long-term data that reveals the potential loss of both these giant sea anemones and their anemonefish from Israeli coral reefs in the near future. The present study further shows that populations of these 2 host species have dwindled to the point that their demographic size patterns and those of their obligate fish associates have become distorted from those observed in more stable populations (Chapter 2).

The sea anemone population sizes reported here were the lowest to date at this site, and represent a mere 39% and 17% of the respective *E. quadricolor* and *H. cripisa* population sizes observed during their peak numbers in 1997 (Chapter 2). The numbers observed in 2014 equate to only 1 *E. quadricolor* for every 306 m<sup>2</sup> of reef area, and 1 *H. cripisa* for each 611 m<sup>2</sup>, similar to their low abundances on mid-shelf reefs in the Great Barrier Reef of Australia (Scott and Baird 2014), but much lower than those at nearby Aqaba, Jordan (only a few kilometers across the Gulf of Aqaba, Dixon et al. 2014). Such low abundances likely cause low rates of successful sexual reproduction, because the anemones are spread out leading to low fertilization rates of their spawned gametes (Levitan 1991). This currently very low local abundance likely drastically affects the local recruitment of anemones, and may explain in part why the smallest size classes here contain much lower-than-expected proportions of individuals during both 2013 and 2014 (compared to those observed when anemones were relatively abundant, Chapter 2).

In the past at this site (during 1996-2000), the size class that exhibited the most individuals was always the class that represented either the largest or smallest sea anemones, with the only exception being when *H. crispa* had a higher number of individuals in the middle size class in 1996 (Chapter 2). The highest number of individuals was found in the middle size class for both species in 2013, and for *H. crispa* in 2014. These results may indicate there was an extended period of reduced recruitment into the smallest size class (see above) during the several years prior to 2013, and less individual growth leading up to the recent censuses, as would be expected for relatively unstable anemone populations (Chapter 2).

Long-term decline in sea anemone abundance negatively impacts the organisms that depend on them for habitat and protection. However, the mutualistic nature of the anemonefish-sea anemone symbiosis also was expected to positively affect, or at least alleviate, some of the hosts' population decline. The rapid loss of individuals during 1996-2000 projected that both sea anemones would become locally extinct in 10-18 years, but this trend appeared to remain accurate only for the less-preferred host *H. crispera*, which mostly hosts juvenile anemonefish. While anemonefish provide a variety of benefits to their host anemones (Chapter 2), the most indispensable is defense from predators. Larger anemonefish are able to be more aggressive toward potential anemone predators, and thus to provide better protection for their host anemones than are small fish. As *E. quadricolor* is the preferred host of *A. bicinctus* in the northern Red Sea, their anemonefish associates were more commonly adults, which are larger and enhance anemone survival (Porat and Chadwick-Furman 2004). The *E. quadricolor* population not only avoided local extinction in 2010 as had been projected, but also exhibited higher numbers than *H. crispera* in 2013 and 2014, reflecting their lesser decline. Thus, the density-dependent process of competition among the anemonefish for hosts, resulting in the relatively larger body size of the remaining fish symbionts as the host habitat area declined, probably has reduced the rate of population decline in the preferred host species.

The survival of host sea anemones may also be attributed to their location of attachment. Individuals of *E. quadricolor* appeared to prefer hard substrates such as overhanging rocks and dead corals, which typically had some level of rugosity for the attachment of the anemone pedal discs deep inside protective crevices. The sea anemone

tentacle crown and body column quickly contract when tentacles or the oral disc is disturbed. When attacked by predators, contraction into hard, protective structures like rocks and dead coral would reduce the amount of lost tissue. While some sea anemones were observed to settle into the sand, the vast majority of the individuals present during 2013-2014 were not. The fact that a relatively larger proportion of *H. crispa* individuals were observed in the sandy substrate may allude to a prey preference by anemone predators. If butterflyfish or sea turtles preferred to eat *E. quadricolor* over *H. crispa*, a lower proportion of *E. quadricolor* would be expected to be observed attached to the less protective sandy substrate. Anecdotal observations indicate that sea turtles are able to extract contracted clownfish anemones more easily from sand than from hard reef areas (personal communication, M. Arvedlund). A statistical analysis of the factors that contribute to sea anemone survivorship at this site found a low dependence upon substrate type, but this may have been in part because so few anemones occur only on sand (Wu et al. 2015). The high proportion of anemones attached to hard, protective substrates at this site is also likely why virtually no substantial anemone motility was observed from 1996-2000 (Chapter 2). In studies where giant sea anemones were observed to move extensive distances (Mitchell 2003), most individuals were attached to sandy substrate with little protection from wave-borne debris, which can compel anemones to move in search of an alternate, less disruptive location.

While no significant difference was observed in anemone body size between 2013 and 2014, the drop observed in body size of the largest anemones within Size Class III for both species could mean fewer gametes produced, which would exacerbate population decline. Still, future mean anemone sizes may be headed in opposite directions, as

indicated by the most recent census data showing that *E. quadricolor* are larger on average and *H. crispa* are smaller on average, compared to their initial sizes in 1996. The change in mean size of anemonefish likely contributed to the opposing trends of body size for the 2 anemone species when compared to 1996. The average size of the anemonefish hosted by *E. quadricolor* had a fairly linear increase ( $R^2 = 0.73$ ) from 1996-2014 (Table 3.1A), while that of fish in *H. crispa* increased less dramatically, and ultimately included relatively small fish. The mean sum of fish lengths in each anemone also increased somewhat linearly ( $R^2 = 0.71$ ) from 1996-2014, but fluctuated for *H. crispa*, indicating that *E. quadricolor* individuals were more regularly occupied by not only larger fish, but also experienced increasing fish biomass that would contribute to supplemental nitrogen and oxygenation, and thus likely more growth as time progressed.

The decline in sea anemone populations negatively affected the number of anemonefish present at the study site. Clownfish sea anemones regularly host more than 2 anemonefish per individual in many places in the Indo-Pacific (Porat and Chadwick-Furman 2004, Fautin and Allen 1997), but the anemonefish observed at this site from 1996-2014 did not appear to alter their group structures by allowing additional anemonefish into their anemones to accommodate for declining anemonefish habitat. As anemonefish must be associated with a host sea anemone in order to survive, less available host space resulted in the parallel declines in anemonefish. The decline in available space in anemones is likely the leading cause for the increase in mean size of hosted anemonefish, as the larger, more dominant anemonefish would more successfully secure anemone space for themselves. The distribution of adult and juvenile anemonefish among the 2 anemone species during 2013-2014 followed the expectation that the

preferred *E. quadricolor* would host most of the adult anemonefish. The adults, as larger individuals, enhanced the survival of their host anemones, which is why *E. quadricolor* seemed to be doing better overall than *H. crispa* at this site.

The proportion of *E. quadricolor* anemones that hosted anemonefish breeding pairs tripled over the 18-year period from 1996-2014. The number of breeding pairs, however, remained relatively similar except for a decrease from 1998-1999. While the reproductive potential was present for anemonefish during the recent years, successful local recruitment of larval anemonefish was likely low, as anemonefish population size was extremely space-limited. These observations recapitulate the need for more sea anemone demographic studies to further understand how the population structure and dynamics of these mutualistic organisms affect each other, as well as the other organisms that depend on sea anemone hosts (e.g., anemoneshrimps and endosymbiotic microalgae). If these sea anemone populations continue to decline at the rates observed, the parallel decline of anemonefish will become compounded, as little space would be available for juvenile recruits or for juveniles waiting their turn to emigrate to the preferred *E. quadricolor* from the less-preferred host *H. crispa*, to form new breeding pairs (Huebner et al. 2012).

This study is the first to report on the dynamics of clownfish sea anemones over multi-year periods, and the effects on their obligate anemonefish population structure. A number of studies have described one-time surveys of clownfish sea anemone abundance and habitat (see Chapter 2), but effective management of these mutualistic organisms requires more detailed analyses of their demographic patterns, including the concomitant demographics of associated mutualists. Some management recommendations have been

made based on the populations at Eilat (Chapter 2), but additional demographic studies would form a more comprehensive understanding of clownfish sea anemone population dynamics across the Indian and Pacific Oceans. These studies would not only increase anemone demographic knowledge where it is deficient, but would also be able to improve the only management recommendations that currently exist for clownfish sea anemones (as summarized in Chapter 2). Unless sea anemone growth and recruitment is augmented on Israeli coral reefs, the 2 clownfish anemone species—and their obligate anemonefish associates—will likely become locally extinct in the near future. Recent small improvements in coral reef condition in Israel may bode well for some increase in sea anemone recruitment, as the recruitment of a number of stony coral species has recently indicated a slight rise on nearby reef areas (Schlesinger and Loya 2013). Additionally, the benefits of the anemonefish mutualism to the host sea anemones—especially *E. quadricolor*—could possibly allow these anemones to maintain a local population beyond the projected trends.

## References

- Allen, G.R., Kaufman, L., Drew, J.A. (2008) *Amphiprion barberi*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, and Samoa. *Aqua, International Journal of Ichthyology* 14(3):105-114.
- Allen, G.R., Drew, J., Fenner, D. (2010) *Amphiprion pacificus*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, Samoa, and Wallis Island. *Aqua, International Journal of Ichthyology* 16(3):129-138.
- Chadwick, N.E., Arvedlund, M. (2005) Abundance of giant sea anemones and patterns of association with anemonefish in the northern Red Sea. *Journal of the Marine Biology Association of the United Kingdom* 85:1287-1292.
- Chadwick, N.E., Ďuriš, Z., Horká, I. (2008) Biodiversity and behavior of shrimps and fishes symbiotic with sea anemones in the Gulf of Aqaba, northern Red Sea. In: Por FD, editor. *The Improbable Gulf: History, Biodiversity, and Protection of the Gulf of Aqaba (Eilat)*. Jerusalem: Magnes Press, Hebrew University. 209–223.
- Fautin, D.G., Allen, G.R. (1997) *Anemonefishes and their Host Sea Anemones: A Guide for Aquarists and Divers*. Revised edition. Western Australian Museum, Perth, 160 pp.
- Fricke, H.W. (1983) Social control of sex: field experiments with the anemonefish *Amphiprion bicinctus*. *Zeitschrift Für Tierpsychologie* 61:71–77.
- Huebner, L.K., Dailey, B., Titus, B.M., Khalaf, M., Chadwick, N.E. (2012) Host preference and habitat segregation among Red Sea anemonefish: effects of sea anemone traits and fish life stage. *Marine Ecology Progress Series* 464:1-15.
- Levitán, D.R. (1991) Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. *The Biological Bulletin* 181(2):261-268.
- Mitchell, J.S. (2003) Mobility of *Stichodactyla gigantea* sea anemones and implications for resident false clown anemonefish, *Amphiprion ocellaris*. *Environmental biology of fishes* 66(1):85-90.
- Porat, D., Chadwick-Furman, N.E. (2004) Effects of anemonefish on giant sea anemones: expansion behavior, growth, and survival. *Hydrobiologia* 531:513–520.
- Schlesinger, T., Loya, Y. (2013) Coral Recruitment and Mortality in the Coral Reefs of Eilat (Red Sea): Indicators of Coral Reef State of Health and Resilience. Oral presentation at the 8th International Conference on Coelenterate Biology, Eilat, Israel, 1-5 December 2013.

- Scott, A., Malcolm, H.A., Damiano, C., Richardson, D.L. (2011) Long-term increases in abundance of anemonefish and their host sea anemones in an Australian marine protected area. *Marine and Freshwater Research* 62(2):87-196.
- Scott, A., Baird, A.H. (2014) Trying to find Nemo: low abundance of sea anemones and anemonefishes on central and southern mid-shelf reefs in the Great Barrier Reef. *Marine Biodiversity* 45(2):327-331.
- Shuman, C.S., Hodgson, G., Ambrose, R.F. (2005) Population impacts of collecting sea anemones and anemonefish for the aquarium trade in the Philippines. *Coral Reefs* 24:564-573.
- Wu, B., McVay, M.J., Billor, N., Chadwick, N.E. (2015) Environmental predictors of long-term growth and shrinkage in the clownfish sea anemones *Entacmaea quadricolor* and *Heteractis crispa*. Manuscript in preparation.

**Table 3.1.** Decline of *Entacmaea quadricolor* (A) and *Heteractis crispa* (B) sea anemones and *Amphiprion bicinctus* anemonefish on coral reefs at Eilat, Israel, northern Red Sea from 1996-2014. Sea anemones were measured by tentacle crown surface area (TCSA), and anemonefish were measured by total length (TL). Values represent means  $\pm$  1 S.D.

**A. *Entacmaea quadricolor***

Year	N	N/10m <sup>2</sup>	Mean TCSA	# Fish	Mean # Fish/Anemone	Mean Fish TL (cm)	Mean Sum of Fish Lengths
1996	88	0.080	202.7 $\pm$ 209.5	124	1.41 $\pm$ 0.87	5.10 $\pm$ 2.34	6.99 $\pm$ 4.13
1997	93	0.085	259.9 $\pm$ 193.7	110	1.24 $\pm$ 0.75	5.72 $\pm$ 2.49	7.43 $\pm$ 4.38
1998	80	0.073	177.3 $\pm$ 132.7	83	1.24 $\pm$ 0.43	5.79 $\pm$ 2.68	7.19 $\pm$ 4.69
1999	74	0.067	156.4 $\pm$ 157.3	99	1.22 $\pm$ 0.77	5.62 $\pm$ 2.46	7.86 $\pm$ 4.30
2000	66	0.060	210.6 $\pm$ 201.4	96	1.45 $\pm$ 0.61	6.47 $\pm$ 2.42	9.02 $\pm$ 4.94
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
2013	52	0.047	270.6 $\pm$ 220.7	73	1.40 $\pm$ 0.53	6.56 $\pm$ 2.28	8.95 $\pm$ 5.13
2014	36	0.033	278.1 $\pm$ 180.1	51	1.42 $\pm$ 0.60	6.99 $\pm$ 2.71	9.71 $\pm$ 6.34

**B. *Heteractis crispa***

Year	N	N/10m <sup>2</sup>	Mean TCSA	# Fish	Mean # Fish/Anemone	Mean Fish TL (cm)	Mean Sum of Fish Lengths
1996	108	0.098	251.8 $\pm$ 165.3	141	1.32 $\pm$ 1.06	2.81 $\pm$ 2.10	3.94 $\pm$ 2.74
1997	109	0.099	255.5 $\pm$ 199.2	111	1.02 $\pm$ 0.79	3.00 $\pm$ 1.63	4.11 $\pm$ 2.27
1998	106	0.096	205.1 $\pm$ 135.4	84	0.90 $\pm$ 0.59	3.05 $\pm$ 1.54	3.39 $\pm$ 1.90
1999	90	0.082	163.7 $\pm$ 129.5	79	0.83 $\pm$ 0.73	3.13 $\pm$ 1.94	3.83 $\pm$ 2.82
2000	92	0.084	203.3 $\pm$ 190.0	109	1.25 $\pm$ 0.79	4.38 $\pm$ 1.91	5.69 $\pm$ 3.25
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
2013	37	0.034	208.8 $\pm$ 116.0	42	1.14 $\pm$ 0.96	4.72 $\pm$ 1.89	6.23 $\pm$ 2.77
2014	18	0.016	220.3 $\pm$ 102.0	17	0.94 $\pm$ 0.70	3.47 $\pm$ 1.28	3.93 $\pm$ 1.51

**Table 3.2.** Variation in mean tentacle crown surface area within Size Classes I-III among years 2013-2014 for *Entacmaea quadricolor* (A) and *Heteractis crispa* (B) on coral reefs at Eilat, Israel, northern Red Sea.

**A. *Entacmaea quadricolor***

Year	Size Class		
	I	II	III
2013	72.7	228.6	600.3
2014	73.4	192.3	436.9
	$X^2 = 4.26$	$df = 2$	$p = 0.12$

**B. *Heteractis crispa***

Year	Size Class		
	I	II	III
2013	103.7	229.2	429.8
2014	81.1	211.1	360.1
	$X^2 = 1.05$	$df = 2$	$p = 0.59$

**Table 3.3.** Variation in the number of sea anemones in each of 2 species (bulb-tentacle anemones *Entacmaea quadricolor* versus leathery anemones *Heteractis crispa*) that hosted differing levels of anemonefish *Amphiprion bicinctus* during 2 years on coral reefs at Eilat, Israel, northern Red Sea. Note that the proportion of anemones that hosted each type of fish category differed significantly between the 2 host species, during both years.

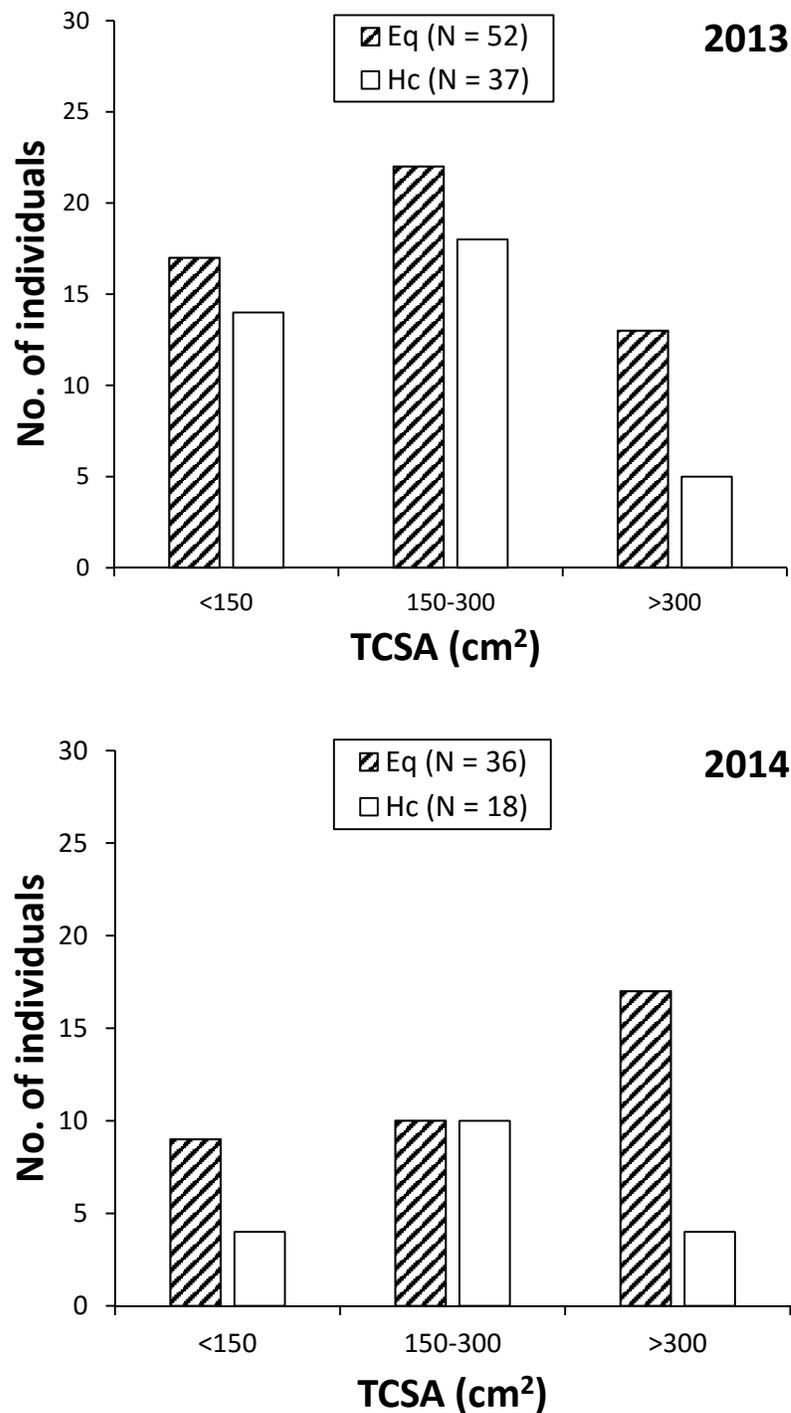
Year & Host Anemone Species	Number of Anemones	Number of anemones that hosted:		
		No fish	Juveniles only	At least 1 adult
<b>2013</b>				
<i>E. quadricolor</i>	52	0	18	34
<i>H. crispa</i>	37	9	17	11
		<b><math>X^2 = 18.79</math></b>	<b><math>df = 2</math></b>	<b><math>p = &lt; 0.001</math></b>
<b>2014</b>				
<i>E. quadricolor</i>	36	1	10	25
<i>H. crispa</i>	18	4	13	1
		<b><math>X^2 = 20.64</math></b>	<b><math>df = 2</math></b>	<b><math>p = &lt; 0.001</math></b>

**Table 3.4.** Variation in the number of anemonefish *Amphiprion bicinctus* resident in each of 2 host species (bulb-tentacle anemones *Entacmaea quadricolor* versus leathery anemones *Heteractis crispa*) between years, at the study site on coral reefs at Eilat, Israel, northern Red Sea. Note that a significantly higher proportion of fish resided in *E. quadricolor* than in *H. crispa* during both years, and that fish abundance (especially of adults) declined more precipitously in the latter than in the former host species.

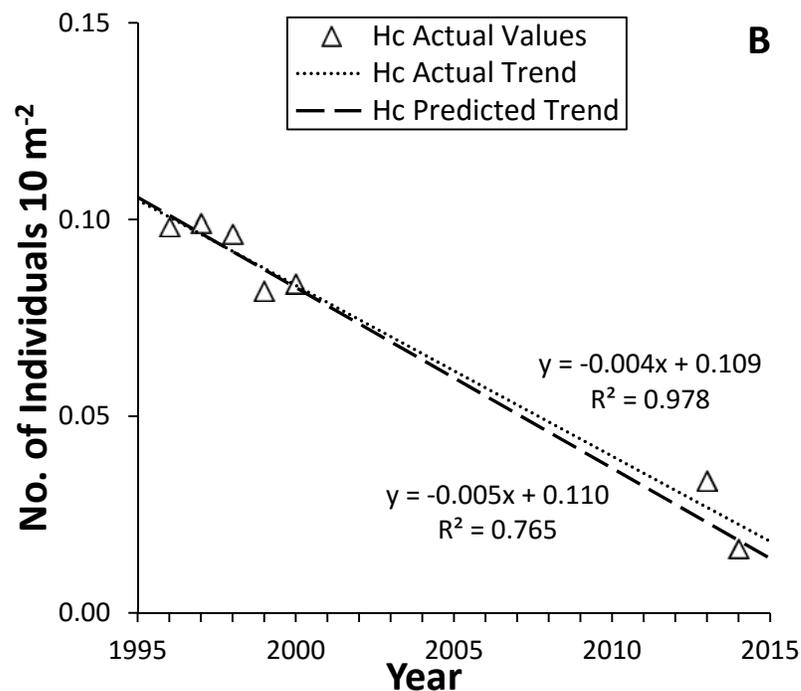
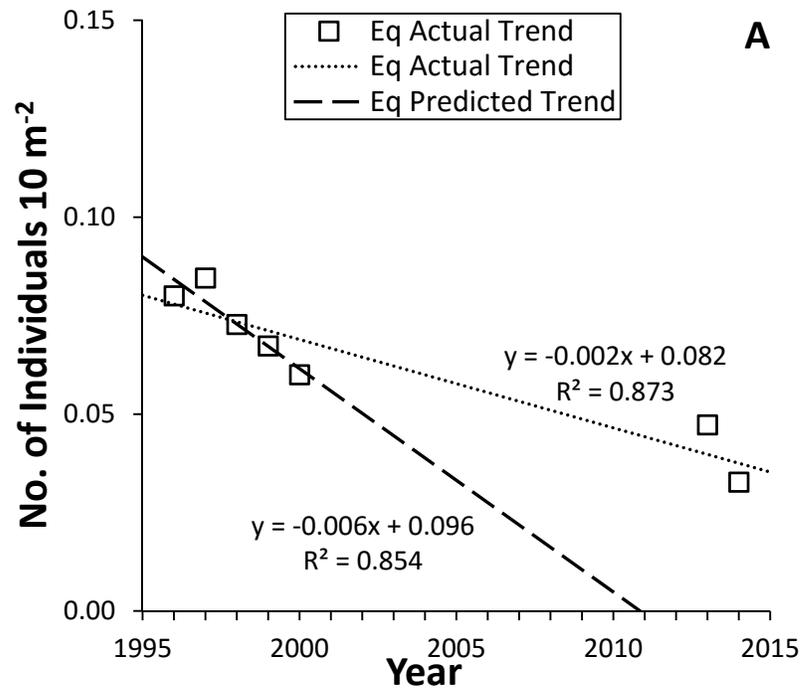
Year & Host Anemone Species	Number of Fish	Number of fish that were:	
		Juveniles	Adults
<b>2013</b>			
<i>E. quadricolor</i>	71	25	46
<i>H. crispa</i>	37	26	11
		<b><math>X^2 = 12.00</math></b>	<b><math>p = &lt; 0.001</math></b>
<b>2014</b>			
<i>E. quadricolor</i>	50	14	36
<i>H. crispa</i>	16	15	1
		<b><math>X^2 = 21.27</math></b>	<b><math>p = &lt; 0.001</math></b>

**Table 3.5.** Breeding pairs of *Amphiprion bicinctus* anemonefish hosted by bulb-tentacle *Entacmaea quadricolor* sea anemones from 1996-2014 on coral reefs at Eilat, Israel, northern Red Sea. Sea anemones were measured by tentacle crown surface area (TCSA) Values represent means  $\pm$  1 S.D. Note the increase in proportion of breeding pairs hosted by *E. quadricolor*.

Year	Number of Anemone Hosts	Mean TCSA of Anemones	% of Anemones Hosting Breeding Pairs
1996	9	445.0 $\pm$ 413.7	10.2
1997	10	380.4 $\pm$ 200.3	10.8
1998	6	355.1 $\pm$ 166.8	7.5
1999	8	319.4 $\pm$ 310.8	10.8
2000	13	481.6 $\pm$ 217.6	19.7
⋮	⋮	⋮	⋮
2013	12	499.5 $\pm$ 250.1	23.1
2014	11	443.9 $\pm$ 172.1	30.6



**Figure 3.1.** Variation in the population size structure of 2 species of clownfish sea anemones, the bulb-tentacle anemone *Entacmaea quadricolor* (Eq) and the leathery anemone *Heteractis crispa* (Hc), between 2 census years (2013 and 2014) on coral reefs at Eilat, Israel, northern Red Sea. Note the decline in abundance in almost all size classes for both species, between years.



**Figure 3.2.** Variation in actual versus predicted values over 20 years at Eilat, Israel, northern Red Sea, in the abundance of 2 clownfish sea anemones: A. Bulb-tentacle anemones *Entacmaea quadricolor* (Eq) and B. leathery anemones *Heteractis crispa* (Hc). Note that the actual rate of decline in Eq abundance by 2013-14 was slower than that predicted from the earlier 1996-2000 trend, while in Hc, the 2 rates of decline (linear slopes) differed little (see text for details).