Nemo no more? Spatial demographic and population genetic analyses of the two-band anemonefish, Amphiprion bicinctus, in the Gulf of Eilat, Red Sea

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NEMO NO MORE? SPATIAL DEMOGRAPHIC AND POPULATION GENETIC ANALYSES OF THE TWO-BAND ANEMONEFISH, *AMPHIPRION BICINCTUS*, IN THE GULF OF EILAT, RED SEA

A Thesis
presented in partial fulfillment of requirements
for the degree of Master of Science
in the Department of Biology
The University of Mississippi

by

JACOB S. HOWELL

August 2016
ABSTRACT

Anemonefishes' obligatory mutualism with sea anemones dictates their occurrence on coral reefs. I examined spatial distribution, settlement, habitat usage, and survival patterns of the two-band anemonefish, *Amphiprion bicinctus*. In a 300 X 30 m study site off the coast of Israel in the Gulf of Eilat, fish and anemone populations were monitored for 13 censuses from October 1996 to August 1997. Based on size, anemonefish were categorized as adults, juveniles, or settlers. Settlers tended to cluster together but displayed significantly dispersed distributions in relation to adult individual fish and breeding pairs. Adult and juvenile anemonefish associated more with, and exhibited higher survival in, *Entacmaea quadricolor*. Settlers primarily inhabited *Heteractis crispa* and exhibited similar survival rates in the two anemone species. From 1997 to 2015, anemone and anemonefish numbers plummeted by 86% and 73%, respectively. In 2015, all 27 remaining anemones were occupied, with most *E. quadricolor* inhabited by adults. This saturated habitat could hinder new anemonefish individuals from settling. These results indicate that if the anemone population does not recover, the anemonefish could face local extinction.

Additionally, due to the sedentary nature of adult anemonefishes, pelagic larval phases represent the only life stage during which dispersal among reef areas may be possible. In order to examine potential dispersal and population genetic patterns of *A. bicinctus* within the Gulf of Eilat, fin clips were collected from anemonefish near Eilat, Israel and Aqaba, Jordan. DNA sequence data was obtained through restriction-site associated DNA (RAD) sequencing to allow for the identification of single-nucleotide polymorphisms (SNPs). I did not observe any self-recruitment
in the Gulf of Eilat based on parentage and relatedness analyses. The Israeli and Jordanian sites were panmictic and also showed signatures of elevated inbreeding levels. While no recent bottleneck was detected, Tajima’s Neutrality Test suggested a population expansion. Such an expansion could be the result of expansion from refugia in the southern Red Sea after the last glacial maximum. The results from both of these studies have management implications for the continued survival of *A. bicinctus* in the Gulf of Eilat.
DEDICATION

To my friends and family who kept me sane over the last three years.
### LIST OF ABBREVIATIONS AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>C#</td>
<td>Census number</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>RAD-seq</td>
<td>Restriction-site associated DNA sequencing</td>
</tr>
<tr>
<td>SNP</td>
<td>Single-nucleotide polymorphism</td>
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<tr>
<td>ddRAD-seq</td>
<td>Double digest restriction-site associated DNA sequencing</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>MAF</td>
<td>Minority allele frequencies</td>
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<tr>
<td>MCMC</td>
<td>Markov chain Monte Carlo</td>
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<tr>
<td>K</td>
<td>Number of putative populations</td>
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<tr>
<td>DAPC</td>
<td>Discriminant analysis of principal components</td>
</tr>
<tr>
<td>N&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Effective population size</td>
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<tr>
<td>I.A.M.</td>
<td>Infinite Allele Model</td>
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<tr>
<td>LOD</td>
<td>Log-likelihood</td>
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<tr>
<td>D</td>
<td>Simpson’s index of diversity</td>
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<tr>
<td>BIC</td>
<td>Bayesian information criteria</td>
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<tr>
<td>N</td>
<td>Sample size</td>
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<tr>
<td>N&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Average number of alleles</td>
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<tr>
<td>H&lt;sub&gt;o&lt;/sub&gt;</td>
<td>Observed heterozygosity</td>
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<tr>
<td>H&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Expected heterozygosity</td>
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ACKNOWLEDGEMENTS

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I. SEA ANEMONE MUSICAL CHAIRS AND THE PLIGHT OF THE TWO-BAND ANEMONEFISH, *AMPHIPRION BICINCTUS*

**Introduction**

Although fish swim, many coral reef fish do not venture beyond their coral reef habitat. Furthermore, some coral reef fish reside within a single coral head (Fishelson 1964; Fishelson et al. 1974) and limit their movements to around that coral. Consequently, the number of potentially inhabitable corals and the spatial proximity of these corals, may determine the number of coral dwelling fish, their movements, and their interaction with conspecifics. Similar to coral dwelling fish, anemonefish (Family Pomacentridae) form obligate mutualisms with sea anemone hosts and rarely venture far from their host anemones (Fautin and Allen 1997).

Anemonefish associate with 10 host anemone species, but most anemonefish species exhibit some level of host specificity or preference by associating with only a few anemone species (Fautin and Allen 1997; Srinivasan et al. 1999; Elliott and Mariscal 2001). In some anemonefish species, adult breeding pairs cohabitate with non-breeding juveniles within the same host anemone or group of anemones (Ross 1978b; Fricke 1979; Fautin 1991; Hattori 1991; Fautin and Allen 1997). In these cases, anemonefish group size can increase with anemone size (Ross 1978b; Fricke 1979; Fautin 1992; Elliott and Mariscal 2001; Buston 2003), but anemonefish aggressively defend their territory from conspecifics as host anemones approach their carrying capacities (Fricke 1979; Elliott and Mariscal 2001; Buston 2003). In other anemonefish species, adults and juveniles segregate into different individual anemones or even species of anemones (Fishelson et al. 1974; Chadwick and Arvedlund 2005) the number and size
of sea anemone hosts influence recruitment and the anemonefish population (Fautin and Allen 1997; Richardson 1999; Srinivasan et al. 1999; Buston 2003; Shuman et al. 2005). Although the dispersal ability of the larvae affects anemonefish recruitment (Jones et al. 2008; Planes et al. 2009; Pinsky et al. 2012), the existing adult anemonefish in the population may prevent conspecific recruitment (Ross 1978b; Fricke 1979; Fautin 1991; Hattori 1991; Elliott et al. 1995; Fautin and Allen 1997; Buston 2003). Adult anemonefish may also evict smaller individuals (Buston 2003; Huebner et al. 2012). Therefore, in addition to the number and size of anemones, anemonefish population dynamics may be determined by the spatial distribution of both the host sea anemones and the existing anemonefish in the population. Sea anemone and anemonefish population studies did not take into account the spatial location of inhabited and uninhabited anemones at their study site (e.g. Huebner et al. 2012).

To investigate the influence of the location of host anemones and conspecific anemonefish on anemonefish population dynamics, we followed the spatial distribution of a population of *Amphiprion bicinctus* and newly settled individuals over time. The two-band anemonefish, *A. bicinctus* is one example of an anemonefish species where the adults are most often segregated from the juveniles (Fishelson et al. 1974; Chadwick and Arvedlund 2005). This species is endemic to the Red Sea, Gulf of Aden, and the Chagos Archipelago, and associates with five species of host sea anemones within this range: *Entacmaea quadricolor; Heteractis aurora; H. crispa; H. magnifica;* and *Stichodactyla gigantea* (Fautin and Allen 1997).

In the northern part of the Gulf of Eilat, Red Sea, *A. bicinctus* only inhabits *E. quadricolor* and *H. crispa* (Chadwick and Arvedlund 2005). In this area, adult *A. bicinctus* normally occupy *E. quadricolor* host anemones, either singly or as breeding pairs and only occasionally associate with juveniles in the same anemone (Fishelson et al. 1974; Chadwick and
Arvedlund 2005). Juvenile *A. bicinctus* reside in both host anemone species and can form groups that cluster within a single anemone, normally *H. crispa*, until the fish reach 30-50 mm in length, when they move to the *E. quadricolor* anemones (Fishelson 1970; Fishelson et al. 1974; Huebner et al. 2012). Due to its thin tentacle morphology, *H. crispa* may not provide sufficient protection from predators as the anemonefish grow larger (Chadwick and Arvedlund 2005; Huebner et al. 2012).

We monitored the populations of both host anemones and anemonefish over a 20-year period. As climate change and other natural or anthropogenic disturbances continue to impact coral reefs worldwide (Hoegh-Guldberg et al. 2007; Munday et al. 2008), long-term monitoring efforts will aid in projecting how these systems change over time and respond to disturbances, and in determining if current protection and conservation efforts are effective (Day 2008; Friedlander and Beets 2008; Cardini et al. 2015). For coral dwelling fish, for example, the demise of coral heads due to habitat destruction and global climate change leads to drastic declines in the associated fish populations (Jones et al. 2008; Munday et al. 2008; Lönnstedt and Frisch 2014). Sea anemones and their associated fish may also demonstrate such a pattern (Shuman et al. 2005; Hobbs et al. 2013).

**Materials and Methods**

**Study Site and Initial Populations of Sea Anemones and Anemonefish**

The study site consisted of a 300 m x 30 m area in the Gulf of Eilat (Aqaba) near the Interuniversity Institute for Marine Sciences in Eilat, Israel (29° 30’ N, 34° 56’ E). In October 1996, all potential host sea anemones (*E. quadricolor* and *H. crispa*) were tagged, identified, and their oral disc diameter measured. Their spatial location and depth, from 0 – 15 m depth, within
the study site was noted. Distances between each anemone and every nearest anemone neighbor within $360^\circ$ were measured using compass headings which were incorporated into a map of the study site’s anemone population (Fig. 1). The presence or absence of *A. bicinctus* within each sea anemone was noted, and the size of each anemonefish was recorded. Total lengths of the fishes were determined using the technique described in Pfister and Goulet (1999) as well as estimating from underwater fish models and taped demarcations on a dive slate. Based on its total length, each anemonefish in the population was placed into one of three size categories: adult (> 75 mm), juvenile (45 – 75 mm), or settler (< 45 mm). The anemonefish present in the October 1996 census period constituted the original population.
Figure 1. Map detailing the locations of sea anemones found in the 300 m x 30 m study area. The sea anemones hosted either single adults (●), breeding pairs (♦), juveniles (▼), settlers (■), mixed groups of adults and juveniles or settlers (★), mixed groups of juveniles and settlers (▽), or no fish (○).
Sea Anemone and Anemonefish Censuses

The initial census (C1) was followed by 12 additional ones (C2 – C13), with an average of 22.25 days between censuses, until August 1997. In each census, the presence or absence of anemones in the study site was noted. The number of anemonefish in each anemone was recorded and the fish ascribed to one of the three size classes. Anemonefish that appeared in the population in these subsequent census periods were termed immigrants. The percentage of time anemones were inhabited or uninhabited was calculated. By matching the dates during which the censuses were conducted to their corresponding lunar phase, the lunar phase at settlement was noted. Furthermore, we determined whether immigrants settled more often into inhabited or uninhabited anemones. Additional one-time censuses were conducted in 2001, 2009 and 2015 and data was extracted from a study at the same research site (McVay 2015) for 1998, 1999, 2000, 2013, and 2014. In the 2001 census and the McVay (2015) data, the overall number of anemonefish, breeding pairs, and sea anemones were counted while in the 2009 and 2015 censuses, the number of anemonefish in each size class, the number of breeding pairs and sea anemones were recorded.

In the C1 – C13 censuses, anemonefish abundance was investigated in relation to anemone species, size, and depth of the anemone habitat. For depth analyses, the study site was divided into three depth ranges (shallow: 0 – 5 m, mid: 5.1 – 10 m, and deep: 10.1 – 15 m). Patterns of turnover of anemonefish inhabitants were explored by tracking the number of anemonefish associating with each anemone of both host species through the 13 census periods. Survival percentages of both original and immigrant anemonefish were calculated by dividing the number of days a particular fish was observed by the total number of days remaining until the end of the 13th census from the time they were first observed and multiplying the ratio by 100.
For example, if an anemonefish was first observed in C4 and survived for 244 days through the end of C13, it would be recorded as having 100% survival. The survival of the original fish and immigrants was examined within and between anemone species. In addition, the anemone and anemonefish populations recorded in the August 1997 census (C13) were compared to the populations in the subsequent censuses from our data and McVay (2015) to determine how the anemone and anemonefish populations changed over time.

Average Nearest Neighbor Analysis

Anemone and anemonefish distributions and fish settlement patterns were examined in ArcMap (Esri). Position data of anemones were imported into blank map layers in ArcMap, and each layer was exported as a shapefile (.shp). Shapefiles for anemones hosting anemonefish of each size class in each census were generated and then analyzed using the spatial statistics tools package. The Average Nearest Neighbor Analysis tool within ArcMap was run to determine if the population of anemonefish as a whole, and the three size classes, displayed a clustered, dispersed, or random distribution in the study area. In addition, the distribution of the settlers was analyzed in relation to that of the fish in the other size classes using the nearest neighbor distances obtained by joining the settler size class layers to the layers of the other size classes.

Statistical Analyses

Based on the data type, different statistical tests were performed using R v. 3.1.2 (R Development Core Team 2014). Student’s t-tests were used to compare oral disk diameters, the percent of unoccupied anemones, the number of consecutively unoccupied anemones, and the number of anemonefish between the two anemone species. Linear regressions were used to examine anemone depth, the number of anemonefish (in each anemone species and both species combined), the number of anemonefish in each size class within each anemone species, and the
number of immigrant fish anemones received, all in relation to anemone diameter. To meet the assumptions of normality, the numbers of adult anemonefish were square root transformed prior to determining their relationship to anemone diameter. One-way ANOVAs were used to compare the number of immigrant anemonefish observed in each depth category and the number of anemonefish observed in C13, 2009, and 2015. A two-way ANOVA was used to compare the number of anemonefish of each size class observed in the two anemone species. Tukey HSD post-hoc tests were employed, where appropriate, to determine the source of the significance obtained from the ANOVAs.

When data violated the assumptions of the parametric tests listed above, non-parametric tests were used. Kruskal-Wallis and Mann-Whitney U tests were used to compare the survival rates of the three size classes of anemonefish within each host anemone species in addition to the survival rates of the size classes of the original and immigrant anemonefish within each anemone species. Tukey and Kramer (Nemenyi) post-hoc tests were used to determine the source of the significance in the Kruskal-Wallis tests. G-tests were employed to determine if the number of anemones and anemonefish (total and within each anemone species) was independent of depth, while a Cochran-Mantel-Haenszel test was used to test if the number of anemonefish in each size class was independent of depth. Settlement of newly settled individuals in relation to lunar phase and calendar month was examined using Rayleigh’s tests (Zar 2010).

Results

Sea Anemone Population

During the October 1996 census a total of 205 anemones, 96 *E. quadricolor* and 109 *H. crispa*, were tagged at the study site. Anemones can contract their tentacles and recede within
reef structure making them hard to spot (Godwin and Fautin 1992; Porat and Chadwick-Furman 2004). Consequently, in the subsequent censuses (C2 – C13) an additional 10 *E. quadricolor* and 18 *H. crispa* were located, tagged, and monitored. During the 13 censuses, anemone mortality occurred, resulting in a loss of 19 *E. quadricolor* and 15 *H. crispa*. By August 1997, 199 anemones, 87 *E. quadricolor* and 112 *H. crispa*, remained in the study area. Anemone size, as determined by oral disk diameter, of *E. quadricolor* and *H. crispa* ranged from 4 – 50 cm and 6 – 40 cm respectively, and the oral disk diameter of *H. crispa* (x = 20.58 cm) was significantly larger than that of *E. quadricolor* (x = 17.72 cm; Student’s t test: t_{194} = 2.651, p = 0.009).

*E. quadricolor* and *H. crispa* occupied depth ranges of 1.1 – 13.4 m and 1.1 – 14.4 m, respectively, with abundances of both anemone species decreasing significantly with depth (G test: G_{[6]} = 101.75, p < 0.001). Most *E. quadricolor* occurred in 0 – 5 m (shallow, 40.2%) and 5.1 – 10 m (mid, 40.2%) depths with only 19.6% found between 10.1 – 15 m (deep, Fig. 2a). Over half of *H. crispa* were found in the shallow depths (56.3%) with the remaining 31.9% and 11.8% residing in the mid and deeper depths, respectively (Fig. 2b). In both anemone species, oral disk diameter changed significantly with depth. The oral disk diameters of *E. quadricolor* displayed a parabolic pattern (Fig. 3a, Quadratic Regression: R^2_{[2, 102]} = 0.135, p = < 0.001) while in *H. crispa* the oral disk diameters increased with depth (Fig. 3b, Linear Regression: R^2_{[1, 129]} = 0.045, p = 0.008). The average nearest neighbor distance for the anemones in the population was 2.9 m. The distribution of *E. quadricolor* was significantly clustered during the first four censuses (C1 – C4). As additional anemones were found and others disappeared, the distribution became random during C5 – C13. *H. crispa* was randomly distributed during every census except C4 (Appendix A).
Figure 2. The mean number of sea anemones (S) and anemonefish (F) at the study site. (a) *Entacmaea quadricolor* and (b) *Heteractis crispa* (hashed bars) and *Amphiprion bicinctus* adults (white), juveniles (grey), and settlers (black) bars, respectively observed in shallow (0-5 m), mid (5.1-10 m) and deep (10.1-15 m) depths.
Figure 3. Oral disk diameter of (a) *Entacmaea quadricolor* and (b) *Heteractis crispa* at the study site. Black, grey, and white circles indicate shallow (0 – 5 m), mid (5.1 – 10 m), and deep (10.1 – 15 m) depths, respectively.

On average, most (81.8) of the anemones in the study area were inhabited by anemonefish at some point during the 13 initial census periods (Student’s t test: $t_{[24]} = 57.607$, $p < 0.001$). While almost every anemone (94.9%) hosted anemonefish during at least one census, only 49.3% of those were occupied during every census they were observed. Of the unoccupied anemones, significantly fewer *E. quadricolor* were unoccupied ($\bar{x} = 10.1\%$; Student’s t test: $t_{[24]} = 11.16$, $p < 0.001$) and for shorter periods of time ($\bar{x} = 0.91$ consecutive censuses; Student’s t test: $t_{[244]} = 4.572$, $p < 0.001$) than *H. crispa* ($\bar{x} = 24.7\%$ unoccupied; $\bar{x} = 2.54$ consecutive censuses). Additionally, of the 12 anemones that never hosted anemonefish, only one was *E. quadricolor* while 11 were *H. crispa*. The uninhabited anemones were randomly distributed throughout the study site in every census except C11 when they were clustered (Appendix B).
On the other hand, the distribution of uninhabited *E. quadricolor* relative to their nearest neighbor that hosted an adult fish was significantly dispersed in C1, random from C2 – C3, and significantly clustered from C4 – C13 (Appendix C). For uninhabited *H. crispa*, the distribution fluctuated between significantly dispersed and random (Appendix C). Anemones that lost fish from one census to the next fluctuated between clustered, random, and dispersed distributions in relation to anemones that gained fish. The bulk of the anemones that gained or lost fish were *H. crispa*. In only three censuses was the number of *E. quadricolor* that gained or lost fish greater than that of *H. crispa*. In those censuses when *E. quadricolor* that gained fish outnumbered *H. crispa*, it was only by one or two anemones.

Anemonefish Population

The anemonefish population in the initial census (C1) consisted of 197 individuals, 17 adults, which included 5 breeding pairs, 43 juveniles, and 137 settlers inhabiting 159 anemones. The total number of fish increased from the 197 fish in the first census in early October (C1) to a maximum of 261 fish in early January (C5; Fig. 4). This increase in the fish population was driven by settlement. Although recruitment occurred year-round, most recruitment occurred from October – December (C1 – C4) with very little occurring during the spring and summer months (Fig. 4, Rayleigh Test: $Z = 101.702$, $p < 0.001$). Furthermore, most recruitment occurred around the new and full moons (Rayleigh Test: $Z = 14.380$, $p < 0.001$). Following the recruitment pulse and mortality of some of the settlers, by mid-August (C13) the population included 195 fish. Due to the growth of fish from one size class to the next, the 195 anemonefish remaining in C13 consisted of 52 adults, which included 11 breeding pairs, 76 juveniles, and 67 settlers (Fig. 4), inhabiting 149 anemones.
Figure 4. Number of *Amphiprion bicinctus* during the 13 census periods from October 1996 to August 1997. The total number of anemonefish (●), adults (□), juveniles (■), and settlers (○) are depicted.

The mean number of anemonefish associating with the two anemone species was not significantly different with *H. crispa* hosting an average of 115.39 ± 4.27 fish per census while *E. quadricolor* hosted 110.00 ± 1.35 fish per census (Student’s t test: t_{14} = 1.202, p = 0.249). On the other hand, fish in the adult, juvenile, and settler size classes inhabited the two anemone species in different ways (ANOVA: F_{2, 72} = 99.15, p < 0.001). Group size increased significantly with oral disk diameter in *E. quadricolor* (Linear Regression: R^2_{1, 101} = 0.042, p = 0.021) but not in *H. crispa* (Linear Regression: R^2_{1, 110} = 0.018, p = 0.081). Adult anemonefish almost exclusively associated with *E. quadricolor* (TukeyHSD: p < 0.001), with the mean number of adults increasing significantly with *E. quadricolor* oral disk diameter (Linear
Regression: $R^2_{[1, 49]} = 0.068, \ p = 0.036$). Juveniles also more frequently associated with *E. quadricolor*, while settlers most often associated with *H. crispa* (TukeyHSD: $p < 0.001$).

Across depths, the average number of fish in each anemonefish size class was significantly different (Cochran-Mantel-Haenszel: *E. quadricolor* $M^2_{[4]} = 108.26, \ p < 0.001$; *H. crispa* $M^2_{[4]} = 98.28, \ p < 0.001$; Fig. 2). The numbers of adults and settlers inhabiting *E. quadricolor* decreased with depth (Fig. 2a). Juveniles associating with *E. quadricolor*, on the other hand, were most abundant in mid depths versus shallow or deep depths. Whilst very few adults inhabited *H. crispa*, those that did were found in shallow waters (Fig. 2b). The numbers of both juveniles and settlers associating with *H. crispa* were highest in shallow waters and decreased with depth (Fig. 2b). Additionally, the number of immigrants that associated with each anemone species decreased with depth, with significantly different numbers in each depth category than expected (Chi-squared Test: $X^2_{[2]} = 8.93, \ p = 0.011$). The number of immigrants that anemones received was not significantly related to the oral disk diameter in either anemone species (Poisson Regression: *E. quadricolor* $z_{[105]} = 0.837, \ p = 0.403$; *H. crispa* $z_{[130]} = 0.375, \ p = 0.708$).

Most adult anemonefish occupied anemones either by themselves or as pairs. In a few instances, single adults or breeding pairs shared an anemone with smaller anemonefish. Single adult fish associated with either a single juvenile or settler in 24 anemones, and with two or three smaller fish in eight and two anemones, respectively. In only four anemones did anemonefish breeding pairs cohabitate with smaller fish. In three of those instances, the additional fish was a settler, and the other involved a breeding pair and a juvenile. Adults or breeding pairs that shared anemones resided in a depth range of 1.1 – 12.2 m and the anemone oral disk diameter ranged from 6 to 40 cm.
During the first census period (C1), adult anemonefish were significantly clustered (Z test: $Z = -2.450$, $p = 0.014$) relative to one another in anemones in the northern half of the study area. The distribution pattern shifted from clustered to random during the next 8 censuses (C2 – C9), with adults associating with anemones throughout the study site. This distribution change was partially due to some adult mortality but primarily driven by the growth of juveniles into the adult size category. In C10, the fish population dynamics led to a significantly dispersed distribution (Z test: $Z = 2.171$, $p = 0.030$), with a return to a random distribution of adult anemonefish for the remainder of the censuses (Appendix D). When only breeding pairs were examined, their distributions throughout the study site were random from C1 – C11. As the number of pairs increased, the distribution changed to significantly dispersed in the C12 and C13 censuses (Z test: C12 $Z = 2.728$, $p = 0.006$; C13 $Z = 2.628$, $p = 0.009$).

Fish in the juvenile size class were randomly distributed in anemones throughout the study site with the exception of the C12 census when juveniles were significantly dispersed (Z test: $Z = 3.588$, $p = 0.003$, Appendix D). On the other hand, in the first four censuses, as settler numbers increased, settlers clustered together (Z test: C1 $Z = -3.119$, $p = 0.002$; C2 $Z = -2.571$, $p = 0.010$; C3 $Z = -2.781$, $p = 0.005$; C4 $Z = -2.167$, $p = 0.030$). As recruitment began to taper off and settlers grew into the juvenile size class or disappeared, the distribution pattern of the remaining settlers became random. C11 was the exception with settlers again exhibiting a significantly clustered distribution within the study site. This clustered distribution was probably due to settlement in less isolated anemones (Z test: $Z = -3.099$, $p = 0.002$; Appendix D).

The spatial patterns of settlers in relation to neighboring adult or breeding pair fish during C1 – C12 and C1 – C13, respectively were significantly dispersed (Appendix E). When settlers were analyzed in relation to their nearest juvenile neighbor, they were significantly dispersed in
C1 (Z test: Z = 6.133, p < 0.001), but were randomly distributed from C2 – C11. In C12 and C13, settlers exhibited a significantly clustered distribution in relation to the juveniles (Z test: C12 Z = -2.183, p = 0.029; C13 Z = -2.905, p = 0.004). When the settlers were examined relative to the adults and juveniles combined, a trend towards increasing clustering was evident. During the first census period, the settlers displayed a dispersed distribution (Z test: Z = 3.624, p < 0.001), but they were randomly distributed during the next five census periods. After C6, the settlers were clustered in relation to the nearest adult or juvenile fish throughout the rest of the census period (Appendix E).

Anemonefish Survival

Adults and juveniles inhabiting *E. quadricolor* had significantly higher survival rates than those associating with *H. crispa* (Mann-Whitney U Test: Adults U = 87.5, p = 0.017; Juveniles U = 1363.5, p = 0.046, Fig. 5). Settler survival rates, on the other hand, did not differ significantly between anemone species (Mann-Whitney U Test: U = 19981.5, p = 0.062). Consequently, mean survival rates of the anemonefish size classes were significantly different in *E. quadricolor* (Kruskal-Wallis Test: $X^2_{[2]} = 20.681$, p < 0.001) but not in *H. crispa* (Kruskal-Wallis Test: $X^2_{[2]} = 5.122$, p = 0.077; Fig. 5). In *E. quadricolor*, both adult (72.1 ± 6.91%) and juvenile (66.2 ± 5.11%) fish displayed significantly higher survival rates than settlers (42.9 ± 3.38%; Tukey and Kramer (Nemenyi): Adults:Juveniles p = 0.792; Adults:Settlers p = 0.002; Juveniles:Settlers p = 0.001; Fig. 5).
Figure 5. Survival rates of *Amphiprion bicinctus* in the host sea anemones *Entacmaea quadricolor* (white) and *Heteractis crispa* (black). Significant differences in anemonefish survival between (*) and within a sea anemone species (letters) are depicted.

The anemonefish initially at the study site (C1) displayed significantly different survival rates in the three size classes (Kruskal-Wallis: $X^2_{[2]} = 11.36, \ p = 0.003$). Adult ($71.4 \pm 9.95\%$) and juvenile ($61.7 \pm 5.38\%$) survival rates were significantly higher than that of the settlers ($44.9 \pm 3.16\%$; Tukey and Kramer (Nemenyi): Adults:Settlers $p = 0.031$; Juveniles:Settler $p = 0.031$) but not significantly different from one another (Tukey and Kramer (Nemenyi): $p = 0.652$). Adults only inhabited *E. quadricolor*, while the survival rates of juveniles and settlers did not significantly differ between the two host anemone species (Mann-Whitney U Test: Juveniles U 403, $p = 0.097$; Settlers U = 2589.5, $p = 0.121$).

On the other hand, looking at just the immigrant anemonefish, there was no significant difference in the survival rates of the three size classes (Adults: $60.9 \pm 1.04\%$; Juveniles: $60.2 \pm$
6.65%; Settlers: 47.3 ± 2.55%; Kruskal-Wallis: $X^2_{[2]} = 3.65$, $p = 0.161$). Adult immigrant anemonefish had significantly higher survival when associating with *E. quadricolor* versus *H. crispa* (Mann-Whitney U Test: $U = 39.5$, $p = 0.037$). On the other hand, survival rates of juvenile and settler immigrants were not significantly different between the two host species (Mann-Whitney U Test: Juveniles $U = 278.5$, $p = 0.211$; Settlers $U = 8281$, $p = 0.322$). Even though a greater number of immigrant fish (179 fish) settled onto already occupied anemones than those without fish (162 fish), those fish that settled onto uninhabited anemones had significantly higher survival rates (55.8 ± 3.31%) than fish settling onto inhabited anemones (44.2 ± 3.24%; Mann-Whitney U Test: $U = 12318.0$, $p = 0.014$).

Follow-up Censuses

The number of anemones at the study site declined. Although anemone numbers increased in 2013 compared to the 2009 census (McVay 2015), anemone numbers dipped to only 27 anemones in 2015 (Fig. 6). The anemonefish followed a similar trend. In 1997 there were 195 anemonefish (Fig. 6). In 2000 and 2013, the anemonefish population increased compared to the previous census, but overall the anemonefish population declined and in 2015 there were a mere 51 fish. Unlike the decline in the total fish population, the number of anemonefish breeding pairs oscillated between six and 13 pairs throughout the years (McVay 2015).

Looking at specific examples, compared to the 1997 census, in 2009, the number of host anemones dropped by 76.4% with only 24 *E. quadricolor* and 23 *H. crispa* left at the study site (Fig. 6). The anemonefish population plummeted 66.7%, with 65 anemonefish inhabiting anemones. In 2009, the anemonefish population consisted of 30 adults (11 breeding pairs), 13 juveniles, and 22 settlers. Unlike in 1997, in 2009 all *E. quadricolor* and 82.6% of *H. crispa* anemones were inhabited with 41 fish associating with *E. quadricolor* and 24 with *H. crispa*. All
of the adult anemonefish but only five settlers inhabited *E. quadricolor* with the remaining 17 settlers residing within *H. crispa*. The juveniles were split between the two anemone species with six fish associating with *E. quadricolor* and seven associating with *H. crispa*.

**Figure 6.** The total numbers of sea anemone hosts (■), *Amphiprion bicinctus* (○), and anemonefish breeding pairs (○) found at the study site. Data points for 1996 and 1997 correspond to census C5 and C13, respectively; census data includes data from McVay (2015).

In 2015, the anemone population further fell to 15 *E. quadricolor* and 12 *H. crispa*. Fifty-two anemonefish inhabited these anemones: 21 adults (8 breeding pairs), 24 juveniles, and 7 settlers. The number of anemones declined by 42.6% from 2009 and 86.4% from 1997 while the number of fish declined by 20.0% from 2009 and 73.3% from 1997. Consequently, in 2015 there were significantly more fish per anemone compared to the 2009 and 1997 censuses (ANOVA: \( F_{[2, 269]} = 19.97, p < 0.001 \)). Furthermore, in 2015, every anemone of both species was inhabited.
Of the 21 adults, the eight anemonefish breeding pairs and three other adults associated with *E. quadricolor*, while only nine of 24 juveniles and no settlers associated with *E. quadricolor*.

**Discussion**

In the Gulf of Eilat, the anemonefish *A. bicinctus* obligatorily inhabits the sea anemones *E. quadricolor* and *H. crispa*. Therefore, anemonefish population size could be constrained by host anemone species preferences and the number, size, and distribution of the host anemones. At the beginning of the study in October 1996, anemonefish inhabited 88.5% of *E. quadricolor* and 67.9% of *H. crispa*. Since most of the uninhabited sea anemones were in a size range that could be inhabited by anemonefish, the sea anemone habitat was not saturated with *A. bicinctus*.

Even though there were fewer *E. quadricolor* than *H. crispa*, more *E. quadricolor* were inhabited demonstrating that the two anemone species were not equivalent habitats. In the Gulf of Eilat, *E. quadricolor* is the preferred host of *A. bicinctus* (Huebner et al. 2012). Indeed, more adult and juvenile *A. bicinctus* associated with *E. quadricolor* than *H. crispa*. Like in previous studies (Chadwick and Arvedlund 2005; Huebner et al. 2012), in our study adult anemonefish, especially breeding pairs, rarely shared an anemone with more than one juvenile or settler. In the instances when sharing did occur, the four breeding pairs that associated with an additional juvenile or settler resided in *E. quadricolor* anemones with a minimum of 30 cm oral disk diameter.

In *E. quadricolor*, group size did increase with oral disk diameter as, in general, the larger anemones of this species were the individuals that hosted more than two anemonefish. As opposed to adults and juveniles, the vast majority of settlers associated with *H. crispa*. Unlike other anemonefish species, such as *A. percula*, which form size hierarchies of the breeding pair
and smaller anemonefish within host anemones (Fautin and Allen 1997), smaller *A. bicinctus* tend to aggregate in the less desirable *H. crispa* anemones before attempting to migrate to nearby *E. quadricolor* (Chadwick and Arvedlund 2005; Huebner et al. 2012).

Anemone size may explain why large fish preferentially inhabit one anemone species over another. Larger anemones can host more or larger anemonefish (Ross 1978b; Holbrook and Schmitt 2005; Mitchell and Dill 2005). In our study, the range of the oral disk diameter of the two anemone species overlapped (Fig. 3), concurring with previously reported anemone oral disk diameters at this site (Chadwick and Arvedlund 2005). On the other hand, the mean oral disk diameter in *H. crispa* was actually significantly larger than that of *E. quadricolor*. Hence anemone size, as reflected by the oral disk diameter, did not explain the anemonefish preference for *E. quadricolor*.

The habitat preference of the different *A. bicinctus* size classes may be influenced by morphological differences between the two anemone species. In the Red Sea, *E. quadricolor* oscillates between bulbous and thick, digitiform tentacle morphs while *H. crispa*’s tentacles are long and thin (Dunn 1981; Chadwick and Arvedlund 2005; Huebner et al. 2012). The *E. quadricolor* digitiform morph has significantly more surface area in their tentacle crowns than *H. crispa* (Huebner et al. 2012), which allows for greater concealment within the tentacle crown, especially for larger-bodied adults. Indeed, in our study, adult and juvenile anemonefish exhibited significantly higher survival rates in *E. quadricolor* than in *H. crispa* (Fig. 5). Settlers, on the other hand, had similar survival rates in both anemone species. When the settlers associating with *H. crispa* grow, their survival may increase if they migrate to *E. quadricolor*, the anemone in which adult survival is higher (Huebner et al. 2012). Movement from *H. crispa*
to *E. quadricolor* may explain the greater observed turnover in *H. crispa* anemones as well as the lack of a consistent distribution pattern of juveniles in our study.

In addition to anemone species, size, and morphology, anemonefish may not inhabit anemones if they are spatially in close proximity to inhabited anemones. An uninhabited ‘halo’ around inhabited anemones may be a consequence of a combination of anemonefish movement and aggression. For example, in large assemblages of *H. magnifica* in the Red Sea, as well as occasionally in the anemones in the Gulf of Eilat, *A. bicinctus* can associate with more than one anemone if the anemones are close enough together, although the fish often retreat to a preferred host when startled or threatened (Brolund et al. 2004; Huebner et al. 2012). In our study, we observed instances of anemonefish moving between two very close anemones. Aggressive adult anemonefish may defend several anemones in close proximity to each other preventing conspecifics from inhabiting these anemones (Allen 1972; Porat and Chadwick-Furman 2004). As more anemonefish in the study site reached adult size, the number of adult anemonefish increased over time, and the distribution of uninhabited *E. quadricolor* became significantly clustered relative to the nearest anemone that hosted an adult fish. The distribution relative to adult-hosting anemone neighbors of uninhabited *H. crispa* fluctuated between significantly dispersed and random. These distribution patterns suggest that adult *A. bicinctus* prevent recruitment not only to the anemones in which they reside but also to the nearby preferred *E. quadricolor* anemones although *H. crispa* may not be actively protected.

The availability of uninhabited anemones may affect *A. bicinctus* recruitment. Similar to previous reports of *A. bicinctus* from the Gulf of Eilat (Fricke 1974) and other anemonefish species (Allen 1972; Ross 1978a; Fautin and Allen 1997; Buston 2004) in our 1996-1997 censuses, anemonefish recruitment occurred year-round. The majority of recruitment occurred
from October to December. During the first 13 censuses, when *A. bicinctus* settled at the study site, despite the availability of uninhabited anemones, and even though these anemones were in close proximity to inhabited ones, more anemonefish settled into inhabited rather than uninhabited anemones. While settlers were significantly clustered as a size class and dispersed from adults, during most of the initial census periods, they were randomly distributed in relation to juveniles. Only during the C12 and C13 censuses did a clustered distribution develop. When the two larger size classes were combined the settlers were significantly clustered in relation to the nearest juvenile and/or adult during large portions of the original 13 census periods. Some of the clustering could be explained by the clustered distribution of the anemone population during the first four censuses. Alternatively, the settlers could be attracted to conspecifics. Larvae of the anemonefish species *A. percula* are attracted to the olfactory cues of conspecifics as they settle onto reefs (Munday et al. 2009). Thus settlers could be attracted to the presence of fellow settlers or juveniles and avoid anemones protected by adults.

Although settling *A. bicinctus* may be attracted to conspecific olfactory cues, we observed significantly lower survival rates for immigrant anemonefish that settled to inhabited anemones versus those that settled to uninhabited anemones. This suggests that either through eviction or stress from aggressive displays conspecifics negatively impact the survival of newcomers. Smaller *A. bicinctus*, like other anemonefish or coral reef fish species (Elliott et al. 1995; Buston 2003; Dirnwöber and Herler 2007; Ben-Tzvi et al. 2009), often experience aggression from or are evicted from habitat patches occupied by larger conspecifics (Moyer and Sawyers 1973; Fishelson et al. 1974; Ross 1978b; Elliott and Mariscal 2001; Huebner et al. 2012).
Even with the differential mortality of immigrants between inhabited and uninhabited anemones, the *A. bicinctus* recruitment and fish survivorship led to a fish population in the August 1997 census that consisted of 52 adults (11 breeding pairs), 76 juveniles, and 67 settlers inhabiting 149 out of the 199 available anemones. The anemone and anemonefish populations dramatically changed between the 1996-1997 censuses and two decades later (Fig. 6). In 2015, the anemone population was a mere 13.6% of the anemone number in 1997, consisting of only 15 *E. quadricolor* and 12 *H. crispa*. The contribution of each anemone species to the total anemone numbers at the study site switched. In 1997, the anemones at the study site consisted of 43.7% *E. quadricolor* and 56.3% *H. crispa* compared to 55.6% *E. quadricolor* and 44.4% *H. crispa* in 2015. The dramatic drop in sea anemone numbers was echoed in the anemonefish population. Even though in the 2000 and 2013 censuses the number of anemonefish were higher than in the preceding 1999 and 2009 censuses, respectively (McVay 2015); overall, in nearly a 20-year period, anemonefish numbers dropped by 73.3% from the 195 fish found in the August 1997 census to 52 in 2015.

Not only did the fish numbers decline, but the fish population demographics changed. In 1997, out of the 195 anemonefish, 26.7% were adults with the breeding pairs accounting for 11.3% of the population. In 2015, 40.4% of the fish population consisted of adults and the breeding pairs were 30.8% of the entire population. Juveniles accounted for 38.9% versus 46.2% in 1997 and 2015, respectively. The largest change occurred in the contribution of the settlers to the population, which changed from 34.4% in 1997 to only 13.4% in 2015. The drop in settler numbers indicates that far fewer new individuals recruited to the population.

Since by 2015 the number of anemones declined by 86.4% from 1997 while the number of fish declined by 73.3% from 1997, there were significantly more fish per anemone compared
to the 1997 census. Furthermore, in 2015, every anemone of both species was occupied. Of the 21 adult anemonefish, 19 adults, which included the eight anemonefish breeding pairs, associated with *E. quadricolor*. Only five *E. quadricolor* did not host adult anemonefish, but these anemones were occupied by seven juveniles with an additional two juveniles cohabitating with two breeding pairs. No settlers associated with *E. quadricolor*. The absence of empty sea anemone habitat will force newly settling anemonefish to interact with, and experience aggression from, their larger conspecifics which may result in high anemonefish settler mortality rates. In addition, our data demonstrates that juvenile anemonefish have significantly higher survival when associating with *E. quadricolor*. Since in 2015 adults dominated this anemone habitat, juveniles, may also encounter aggressive behavior from adults, preventing the juveniles from migrating to this preferred habitat as the fish grow, leading to increased mortality rates.

Anemonefish are obligate sea anemone dwellers, hence, anemonefish survival relies on the existence of suitable sea anemone habitats. When the availability of sea anemone habitat declines due to, for example, collection for the aquarium trade as occurred in the Philippines (Shuman et al. 2005) or bleaching due to climate change (Hobbs et al. 2013), anemonefish populations may be adversely affected. Understanding what affects the sea anemone population is a first step to projecting the future for this symbiosis in this region. The Gulf of Eilat has experienced increased development over the last few decades, with increased inputs of pollution and a decline or loss of reef species (Loya 1975; Fishelson 1995; Rinkevich 2005). Additionally, a rise in diving tourism has negatively impacted coral reefs in the Gulf of Eilat including divers physically damaging coral colonies (Zakai and Chadwick-Furman 2002). Some studies have explored rearing *A. bicinctus* or producing host anemones in captivity with the intent of releasing them onto reefs (Maroz and Fishelson 1997; Scott and Baird 2015), but these restoration efforts
do not address the reason for the decline and hence may not prove successful in the long run. Future studies and management efforts should focus on deciphering the causes of the host anemones’ demise, potentially eliminating these effects and thereby enabling the recovery of the host anemones *E. quadricolor* and *H. crispa*. Hopefully, *A. bicinctus* recruitment and population growth will follow. Otherwise the populations of these anemonefish and their sea anemone hosts may face local extinction.
II. DOES NEMO STAY CLOSE TO HOME?

Introduction

Coral reefs constitute a distinct tropical habitat with a plethora of invertebrate and vertebrate inhabitants, many of which rely on the coral reef structure for their survival. Even on fringing reefs, the coral reef habitat is interspersed with sand and/or algae dominated habitat. For many coral reef species, the adult life stage is sedentary with limited dispersal capability (Horne et al. 2008; Planes et al. 2009; van der Meer et al. 2012). Hence, the breaks in the coral reef continuum may isolate one coral reef stretch from an adjacent one. On the other hand, a pelagic larval phase, which can range from days to months, may enable progeny of coral reef residents to connect their natal reef to adjacent or distant reefs via gene flow (Patterson and Swearer 2007; Planes et al. 2009; van der Meer et al. 2012). Due to this potential for long-distance dispersal, marine populations have historically been considered fairly open in regards to gene flow and dispersal (Caley et al. 1996). On the other hand, self-recruitment may help maintain marine populations (Gerlach et al. 2007; Planes et al. 2009; Saenz-Agudelo et al. 2011; van der Meer et al. 2012).

Understanding how marine populations are connected is key to successful management of imperiled and/or commercially important populations and species (Sale et al. 2005). Factors such as habitat topography (Saenz-Agudelo et al. 2011), patchiness (Cowen et al. 2006; Buston et al. 2012; Pinsky et al. 2012; D'Aloia et al. 2013) and prevailing currents (Johannes 1978; Roberts 1997; Swearer et al. 1999) may influence how and/or to what extent populations of sedentary adult reef species are connected. For example, reef sites isolated from other suitable
reef sites such as those around Kimbe Island, Papua New Guinea, exhibit high levels of self-recruitment of reef fishes (Jones et al. 2005; Almany et al. 2007; Planes et al. 2009) while self-recruitment was lower in less isolated, coastal reef sites that are more easily connected via tidal cycles or currents (Saenz-Agudelo et al. 2011; D’Aloia et al. 2013). Dispersing larvae may also exert control on where they settle (Armsworth et al. 2001; Kingsford et al. 2002; Leis et al. 2011). Coral reef fish larvae have well-developed sensory systems allowing them to orient themselves in the water column (Simpson et al. 2010), locate suitable habitat (Leis et al. 2002; Gerlach et al. 2007; Dixson et al. 2008), and detect conspecifics or predators (Sweatman 1988; Dixson et al. 2010). Additionally, as larvae develop, they can display high swimming speeds (Fisher 2005) as well as sustained swimming abilities (Stobutzki and Bellwood 1997; Fisher and Bellwood 2002) thereby enabling them to influence where they eventually settle.

One group of coral reef fish with adults confined to a very small coral reef area and pelagic larvae are anemonefish. Throughout the Indo-Pacific, ~30 anemonefish species form obligate mutualisms with 10 host sea anemone species (Fautin and Allen 1997). Anemonefishes rely on their host anemones for protection from predators and rarely venture more than a few meters away (Allen 1972; Fautin and Allen 1997). Thus, rather than adult anemonefish moving long distances to feed or spawn, anemonefish reside in pairs or groups within their hosts. When in groups, the two largest fish are the breeding female and male while the remainder are non-breeding juveniles (Fricke 1979; Fautin 1992; Fautin and Allen 1997; Elliott and Mariscal 2001). Breeding pairs lay demersal eggs near the anemone’s base and engage in parental care until the relatively developed larvae hatch (Allen 1972; Fautin and Allen 1997).

During the pelagic larval phase, which lasts between 8-22 days depending on the anemonefish species (Wellington and Victor 1989; Fautin and Allen 1997; Jones et al. 2005;
Nanninga et al. 2015), larvae can travel large distances before settling to a suitable reef habitat. *Amphiprion percula* larvae, for example, traversed up to 35km among a network of marine protected areas in Kimbe Bay, Papua New Guinea (Planes et al. 2009). Thus, if those larvae reach adulthood and successfully breed, the natal and settlement reefs would be connected via gene flow. Long-distance dispersal, on the other hand, does not always occur, and larvae could also settle to their natal reef. At four isolated sites in East Australia, the endemic anemonefish *A. mccullochi*, showed 68-84% self-replenishment, a proxy for self-recruitment (van der Meer et al. 2012). Additionally, some populations can experience both dispersal and significant self-recruitment as evidenced in populations of *A. percula* and *A. polymnus* in Kimbe Bay, Papua New Guinea that demonstrated up to 40% and 32% self-recruitment, respectively, with some individuals settling less than 100m from their birth anemone (Jones et al. 2005; Planes et al. 2009).

*Amphiprion bicinctus*, is an anemonefish species endemic to the Red Sea, Gulf of Aden, and the Chagos Archipelago and inhabits five species of host sea anemones: *E. quadricolor; H. aurora; H. crispa; H. magnifica; S. gigantea* (Fautin and Allen 1997). In the Gulf of Eilat, a northern extension of the Red Sea, *A. bicinctus* resides in only *E. quadricolor* and *H. crispa* (Fishelson 1970; Fricke 1974; Chadwick and Arvedlund 2005). Adults most often occupy *E. quadricolor*, either singly or as breeding pairs (Fishelson 1970; Fishelson et al. 1974; Chadwick and Arvedlund 2005; Huebner et al. 2012), while juveniles are often relegated to *H. crispa* (Fishelson 1970; Fishelson et al. 1974; Chadwick and Arvedlund 2005; Huebner et al. 2012). Populations of *A. bicinctus* occur on both western (Israeli) and eastern (Jordanian) coastlines. Over the past 20 years, the numbers of *A. bicinctus* and it’s host sea anemones near Eilat, Israel have declined by 73% and 86% respectively (Howell et al. 2016). During a year-long censusing
effort in 1996/1997, ample numbers of new individuals recruited to the Israeli site (Howell et al. 2016), but the source of the new individuals is not known and could be from self-seeding or other populations. Few studies have examined population genetics or connectivity in *A. bicinctus* (Nanninga et al. 2014; Nanninga et al. 2015; Saenz-Agudelo et al. 2015) and none at a fine scale in the northern Gulf of Eilat where genetic structuring has been observed in at least one sessile coral species over small geographic distances (Zvuloni et al. 2008).

Directly assessing the source of larval recruitment is practically impossible. Hence, estimates of the source of recruitment are often obtained using genetic or geochemical methods (Cowen and Sponaugle 2009). In this study, we examined connectivity and recruitment of *A. bicinctus* between two populations in the northernmost part of the Gulf of Eilat using restriction-site associated DNA (RAD) sequencing. RAD sequencing is a cost-effective alternative to traditional microsatellites that allows a large number of single-nucleotide polymorphisms (SNPs) throughout the entire genome of the study organisms to be sampled rather than just a few loci (Hohenlohe et al. 2011; Peterson et al. 2012; Graham et al. 2015). Understanding how marine populations are connected and to what extent populations rely on natal larval supplies or dispersal from other locales is key to successful management of imperiled and/or commercially important species or populations (Sale et al. 2005). Thus, knowing how much gene flow is occurring and the source of new recruits can aid potential recovery efforts for *A. bicinctus* in Eilat.

**Materials and Methods**

Sampling and DNA Extraction
Fin clips were taken from 96 anemonefish (48 from Israel and 48 from Jordan) from the Gulf of Eilat. The Israeli samples were taken from a 300 m x 30 m area near the Interuniversity Institute of Eilat (29° 30’ N, 34° 56’ E) while the Jordanian samples were obtained from a site near the Marine Science Station in Aqaba. All known breeding pairs (N = 13) in the Israeli population were sampled.

DNA was extracted from the fin clips following a phenol-chloroform extraction protocol (Goulet and Coffroth 2004). DNA from an additional 8 fin clips was extracted using the Wizard SV Genomic DNA Purification System (Promega). RAD-seq libraries were prepared using a modified version of the ddRAD-seq protocol (Peterson et al., 2012), 3RAD, described in Graham et al. (2015). In separate wells of a 96-well plate, internal i5 and i7 adapters were added and 100 ng genomic DNA from each individual was digested with two restrictions enzymes (EcoRI-HF and NdeI; NEB), while a third restriction enzyme (CviQi; NEB) was used to cleave adapter dimers. First, the samples were incubated for digestion in a thermocycler for ~1 hr at 37°C. The i5 and i7 adapters were then ligated to the digested DNA fragments via incubation in a thermocycler for two cycles at 22°C for 20 min and 37°C for 10 min, then 80°C for 20 min before holding at 20°C. The post-ligation samples were cleaned via SpeedBeads and re-suspended in 20µL TLE. 10 µL of the ligation product was then transferred to a new 96-well plate along with external iTru5 and iTru7 PCR primers (Integrated DNA Technologies). The subsequent PCR amplification reaction was run on a thermocycler at 95°C for 2 min, 20 cycles of: 98°C for 20 sec, 60°C for 15 sec, 72°C for 30 sec, 72°C for 5 min, and then held at 15°C.

The PCR-amplified products were pooled and purified using a QIAquick PCR purification kit (QIAGEN). DNA fragments of 330 – 455-bp were selected and isolated using a Pippin Prep (Sage Science) size selection machine. After size selection, the library pools were
run on an Agilent 2100 Bioanalyzer system for confirmation of the size range obtained from the Pippin. In order to determine the concentration of DNA with correctly attached Illumina primers, qPCR was performed for each sample pool. Samples were sequenced on an Illumina NextSeq.

Processing of Sequence Reads

Raw single-end sequence data were downloaded from Basespace, demultiplexed into .fastq files for each individual anemonefish, and concatenated across lanes and multiple sequencing runs. The barcodes and restriction sites were then trimmed from the sequences resulting in 56-bp fragments for all individuals. Trimmed sequences were then processed using the pyRAD (Eaton 2014) software pipeline. Nucleotide base calls with quality scores below 20 were denoted as “N,” and sequences with more than seven N’s were discarded. Sequences were clustered at 85% similarity within individual samples with sequences then being clustered across samples and aligned. The minimum number of individuals that must be represented for any particular locus to be retained within the final dataset was set at 70. Individuals with low numbers of called loci were removed from the dataset. The resulting dataset contained 3045 loci with 2001 SNPs for 80 individuals (39 from Israel and 41 from Jordan).

Population Genetic and Statistical Analyses

The poppr package (Kamvar et al. 2015) in R v. 3.2.4 (R Development Core R Development Core Team 2014) was used to remove phylogenetically uninformative loci with minority allele frequencies (MAF) of less than 0.01 from the dataset. This analysis removed 1031 loci from the original dataset. All population genetic analyses were run using both the original dataset (2001 loci) and the reduced dataset (970 loci). Individual locus tests for Hardy-Weinberg Equilibrium, Simpson’s diversity index calculations, linkage disequilibrium tests (10,000 permutations of the data were used to generate p-values), and AMOVAs (10,000 permutations
were used to generate p-values) comparing the Israeli and Jordanian populations were also conducted in poppr. Global test for Hardy-Weinberg equilibrium, G tests for population differentiation, allele frequency calculations, F-statistics, and estimates of the effective number of migrants per generation were conducted using GenePop (Raymond and Rousset 1995; Rousset 2008). Population structuring analyses to calculate G_st (100,000 permutations) and expected and observed heterozygosity calculations were conducted in GenoDive (Meirmans and Van Tienderen 2004).

A Bayesian clustering analysis was performed using STRUCTURE version 2.3.4 (Pritchard et al. 2000). The analysis was run under the admixture model with correlated allele frequencies using a burn-in period of 200,000 MCMC iterations and 300,000 iterations per run with K (the number of putative populations) ranging from 1 to 10. For each value of K, the analysis was repeated 5 times. Results were extracted using Structure Harvester (Earl 2012) and the number of clusters were evaluated using ΔK (Evanno et al. 2005). Additionally, a discriminant analysis of principal components (DAPC) was run in the adegenet package in R (Jombart 2008; Jombart and Ahmed 2011) in order to identify clusters by first transforming the data using a principal components analysis.

LDNe v. 1.31 (Waples and Do 2008) was used to estimate effective population size (N_e) for the Israeli and Jordanian populations using the lifetime monogamy reproductive model. These estimates are based on linkage disequilibrium and allele frequency data, and LDNe uses both jackknife and parametric methods for calculating confidence intervals. The allele frequencies cutoff values used in the analysis were 0.05, 0.02, and 0.01. In cases where negative estimates of N_e were reported, the value was interpreted as an infinitely large effective population (Waples and Do 2008).
BOTTLENECK v. 1.2.02 (Cornuet and Luikart 1996) was used to determine if a recent population bottleneck had occurred in this group of *A. bicinctus*. The Infinite Allele Model (I.A.M.), whereby allelic diversity is reduced more quickly than heterozygosity in response to a bottleneck, was used as the model for the analysis. Expected and observed distributions of heterozygosity were computed for each locus in each population in order to determine if loci exhibited heterozygosity excess or deficiency in order to compute p-values. In order to test for a population expansion, Tajima’s $D$ (Tajima 1989) and Fu’s $F_s$ (Fu 1997) tests were conducted using ARLEQUIN v. 3.5.2.2 (Excoffier and Lischer 2010). To determine the validity of a population expansion, mismatch distribution with Harpending’s raggedness index (Harpending 1994) was conducted in ARLEQUIN as well, using pairwise molecular differences and 1000 bootstrap replicates.

Parentage analysis (parent-pair, sexes unknown) was conducted using Cervus v. 3.0 (Kalinowski et al. 2007). Cervus uses a maximum-likelihood approach to predict parentage through calculation of log-likelihood (LOD) scores for each potential parent-offspring pair as well as LOD scores for candidate parent pairs and offspring trios. Critical LOD scores for 95% and 80% (strict and relaxed, respectively) confidence levels are determined through a simulation stage where 200,000 offspring are simulated based on the allele frequencies in the dataset and the number of candidate parents tested for each offspring. In order to corroborate the results from Cervus, additional relatedness analyses were conducted using ML-Relate (Kalinowski et al. 2006), which computes maximum likelihood scores for relatedness. Relatedness values calculated for each pair of individuals can range from 0 – 1 with 0 being unrelated.
Results

Population Genetic Statistics

Results for analyses run using both the original, 2001 locus (2001L) dataset and the reduced, 970 locus (970L) dataset are reported in this section. Of the 2001 loci, 114 (5.7%) and 124 (6.2%) loci from the Israel and Jordan populations, respectively did not conform to Hardy-Weinberg expectations. Since loci were shared between populations, when the populations were combined, 216 (10.7%) rather than 238 loci did not conform to Hardy-Weinberg expectations. Despite these deviations from Hardy-Weinberg equilibrium, there was no significant difference in either the global per locus allele or genotypic frequencies between the Israel and Jordan populations (2001L: Fisher’s Exact G Test: Allele: $\chi^2_{[4002]} = 1844.2$, p = 1.00, Genotype: $\chi^2_{[4000]} = 1538.3$, p = 1.00; 970L: Fisher’s Exact G Test: Allele: $\chi^2_{[1940]} = 1303.6$, p = 1.00; Genotype: $\chi^2_{[1938]} = 1000.6$, p = 1.00). Observed and expected levels of heterozygosity were very low, with the observed values being lower than expected in both Israel and Jordan analyzed together and separately (Table 1). The Simpson’s Index (D) values for Israel, Jordan, and the two populations together were 0.974, 0.976, and 0.988 respectively and identical in both datasets (Table 1) indicating low diversity in the population both within and between sites. $F_{is}$ values for the 2001L dataset were 0.161, 0.197, and 0.178 for Israel, Jordan, and the two populations combined, respectively. $F_{is}$ values were higher in the 970L dataset at 0.225, 0.261, and 0.243 for Israel, Jordan, and the total population respectively. There were no significant levels of linkage disequilibrium in either the individual populations (2001L: Israel: $r_d = 0.001$, p = 1.00, Jordan: $r_d = 0.005$, p = 1.00; 970L: $r_d = 0.001$, p = 1.00; Jordan: $r_d = 0.006$, p = 1.00) or when combining the two (2001L: $r_d = 0.002$, p =1.00; 970L: $r_d = 0.003$, p =1.00).
Table 1: Population genetic statistics describing both the original (2001L) and reduced (970L) datasets. N = sample size; \( N_a \) = the average number of alleles per locus; \( H_o \) and \( H_e \) = observed and expected heterozygosity, respectively; \( F_{is} \) = the inbreeding coefficient; \( D \) = Simpson’s Diversity Index.

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>( N_a )</th>
<th>( H_o )</th>
<th>( H_e )</th>
<th>( F_{is} )</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2001</td>
<td>970</td>
<td>2001</td>
<td>970</td>
<td>2001</td>
<td>970</td>
</tr>
<tr>
<td>Israel</td>
<td>39</td>
<td>1.213</td>
<td>1.278</td>
<td>0.023</td>
<td>0.031</td>
<td>0.028</td>
</tr>
<tr>
<td>Jordan</td>
<td>41</td>
<td>1.185</td>
<td>1.264</td>
<td>0.02</td>
<td>0.029</td>
<td>0.026</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>1.448</td>
<td>1.433</td>
<td>0.022</td>
<td>0.03</td>
<td>0.027</td>
</tr>
</tbody>
</table>
The global pairwise F_{ST} values for the Israeli and Jordanian sites were very low (2001L: 0.003; 970L: 0.004). The G_{st} values were 0.001 and 0.002 for the 2001L and 970L datasets respectively and not significantly different from zero (2001L: p = 0.251; 970L: p = 0.261), thus there was not evidence of population structuring between the two sites. AMOVA results indicated that the majority of the variance seen in the data is within individuals (2001L: 89.8%, \( \Phi_{[80]} = 0.101, p < 0.001; 970L: 82.0\%, \Phi_{[80]} = 0.180, p < 0.001 \)) while between-site variance was low (2001L: -0.10%, \( \Phi_{[1]} = -0.001, p = 0.696; 970L: -0.09\%, \Phi_{[1]} = -0.001, p = 0.696 \)). Negative variance components, like that seen in the between-site comparison, can occur in the absence of genetic structure and most likely reflect that the true variance percentage is zero. The estimated number of migrants between Israel and Jordan, based on the mean frequency of private alleles (2001L: 0.02; 970L: 0.04), was ~7 and 3 \( A. \) bicinctus for the 2001L and 970L datasets respectively.

Clustering Analyses

Individuals from Israel and Jordan were not clustered into separate populations, as demonstrated by the results from STRUCTURE. The clustering analysis suggested \( K = 2 \) populations based on \( \Delta K (\Delta K = 52.75; \) Evanno et al. 2005). When examining the bar plot output (Figure 7) and the Clumpp files that detail the probabilities of individuals being assigned to each of the populations every \( A. \) bicinctus individual had a \( \geq 0.90 \) probability of being assigned to the same population rather than two different populations. Similarly, when the dataset was analyzed using DAPC, 2 clusters were identified based on the Bayesian Information Criteria (BIC) values. Within each of those two clusters, individuals from Israel and Jordan were represented in roughly equal numbers, and formed a single cluster of points when principal components were
plotted (Figure 8). These results suggest that anemonefish from Israel and Jordan are a single population with a high level of gene flow occurring between the sites.

Figure 7. Structure plot showing population assignments for the anemonefish *Amphiprion bicinctus* in the Eilat and Aqaba field sites. Each column represents a single fish. Bars 1 – 39 refer to Israeli fish while bars 40 – 80 refer to Jordanian fish. Different gray and black bar portions represent different genetic clusters (K = 2), and the size of each color bar in a column represents the likelihood that a particular fish would be assigned to that cluster.
Effective Population Size

For both the original and reduced datasets, the estimated effective population size for both populations under the monogamy mating model, ranged from 218 to infinitely large depending on the allele frequency used. This is likely due to the lack of linkage disequilibrium found in these populations (Table 2).
Table 2: Summary of LDN\textsubscript{e} effective population size analysis for both original (2001L) and reduced (970L) datasets. N\textsubscript{e} = effective population size; Inf = an infinitely large population size.

<table>
<thead>
<tr>
<th></th>
<th>2001 Loci</th>
<th>Israel</th>
<th>2001 Loci</th>
<th>Jordan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele Frequencies Used</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>0.02</td>
<td>0.01</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Harmonic Mean Sample Size</td>
<td>24.8</td>
<td>35.1</td>
<td>35.7</td>
<td>27.1</td>
</tr>
<tr>
<td>Overall ( r^2 )</td>
<td>0.029</td>
<td>0.034</td>
<td>0.021</td>
<td>0.04</td>
</tr>
<tr>
<td>Expected ( r^2 )</td>
<td>0.046</td>
<td>0.031</td>
<td>0.03</td>
<td>0.041</td>
</tr>
<tr>
<td>Estimated ( N_e )</td>
<td>-38.8</td>
<td>218.1</td>
<td>1083</td>
<td>-475.7</td>
</tr>
<tr>
<td>95% Confidence Intervals (Parametric)</td>
<td>-48.3 – Inf</td>
<td>197.0 – 243.8</td>
<td>936.9 – 1281.6</td>
<td>96.5 – Inf</td>
</tr>
<tr>
<td>95% Confidence Intervals (JackKnife)</td>
<td>-48.7 – Inf</td>
<td>176.5 – 282.2</td>
<td>711.9 – 2219.6</td>
<td>63.5 – Inf</td>
</tr>
</tbody>
</table>

|                   | 970 Loci |        |           |        |
| Harmonic Mean Sample Size | 24.8      | 35.1   | 35.5      | 27.1   |
| Overall \( r^2 \)  | 0.029     | 0.034  | 0.031     | 0.04   |
| Expected \( r^2 \) | 0.046     | 0.031  | 0.031     | 0.041  |
| Estimated \( N_e \) | -38.8     | 218.1  | 8853      | -475.7 |
| 95% Confidence Intervals (Parametric) | -48.3 – Inf | 197.0 – 243.8 | 2792.5 – Inf | 96.5 – Inf | 363.9 – 598.2 | -1946.8 – Inf |
| 95% Confidence Intervals (JackKnife) | -48.7 – Inf | 176.5 – 282.2 | 1209.6 – Inf | 63.5 – Inf | 293.1 – 956.0 | 192217.5 – Inf |
Population Bottleneck

Population bottlenecks were not identified in either Israel or Jordan. For Israel, the expected number of loci with heterozygosity excess in the original dataset was 547.79, but only 9 were observed while 1332 loci exhibited heterozygosity deficiency (Sign Test: \( p = 0.00 \)). For Jordan, the expected number of loci with heterozygosity excess was 499.35, but only 9 were observed while 1205 loci were deficient (Sign Test: \( p = 0.00 \)).

Neither the Wilcoxon Tests for heterozygosity excess (Israel: \( p = 1.00 \); Jordan: \( p = 1.00 \)) nor the allele frequency distributions (Israel: normal L-shaped distribution; Jordan: normal L-shaped distribution) detected signatures of a recent population bottleneck for either population. The Tajima’s \( D \) test results indicate, rather than a recent bottleneck, the \( A. bicinctus \) population is undergoing an expansion \( (D = -2.82, p = 0.00) \). Similarly, Fu’s \( Fs \) test indicates a population expansion \( (Fs = -15.6, p = 0.003) \). The null hypothesis of a population expansion could not be rejected from the results of the mismatch distribution \( (SSD = 0.003, p = 0.930) \) or the raggedness index \( (r = 0.001, p = 0.820) \).

Parentage Analysis

When only the number of breeding pairs observed in the Israeli population was incorporated into the Cervus simulation, none of the \( A. bicinctus \) offspring were assigned to parent pairs based on their joint LOD scores. Seven offspring were assigned to single parents at the relaxed confidence level \( (\text{Critical LOD: } -24.00) \). Three of the 7 assignments involved \( A. bicinctus \) from Jordan being assigned to Israeli parents while the reverse is true for the remaining 4 assignments. Those 7 assignments disappear when estimates of the number of breeding pairs for the simulation include potential Jordanian breeding pairs. In ML-Relate, each of the sampled anemonefish was paired with every other anemonefish in the dataset to determine the pair’s
relatedness. No parent/offspring relationships were assigned, and each anemonefish pair was
determined to be unrelated. Thus, no evidence of self-seeding was detected in these two
populations, based on these samples, while the potential for direct dispersal between them is
suggested.

Discussion

Characterizing how and to what extent populations are connected is important for their
management and monitoring (Roberts 1997; Sale et al. 2005). In the present study, analysis of
reduced-representation genomic data from the two-band anemonefish, *A. bicinctus*, in the Gulf of
Eilat revealed no population structure between fish from Israel and those from Jordan. The lack
of population structure and the results from clustering analyses indicate that the *A. bicinctus*
populations on both sides of the Gulf of Eilat are actually a single population. The Gulf of Eilat
is quite narrow, and the Israeli and Jordanian sites are not far apart. Given that only a single
migrant per generation is enough to encourage panmixia (Mills and Allendorf 1996), and around
two or seven migrants per generation (depending on the dataset used) were estimated from our
data, it is not surprising that little genetic structuring was observed. These results are consistent
with previous studies examining gene flow of *A. bicinctus* in the Gulf of Eilat and the Red Sea
proper. High gene flow was reported for *A. bicinctus* populations throughout the northern Red
Sea (Nanninga et al. 2014; Saenz-Agudelo et al. 2015). Additionally, the Fst values we report
were very similar to those pairwise values reported by Saenz-Agudelo et al. (2015) for samples
obtained from Saudi Arabian coast of the Gulf of Eilat and other northern Red Sea sites.

Few studies have examined gene flow and population structure within the Red Sea. While
local selection regimes can promote population structuring over small distances, as in the coral,
*Stylophora pistillata*, (Zvuloni et al. 2008), most studies have reported similar levels of panmixia
within the Gulf and Red Sea proper for populations of multiple reef fish species such as rabbitfishes (*Siganus rivulatus* and *S. luridus*) (Hassan et al. 2003), lionfish (*Pterois miles*) (Kochzius and Blohm 2005), and wrasse (*Larabicus quadrilineatus*) (Froukh and Kochzius 2007). Like most reef fish species, these fishes are often tied to a reef area as adults (Sale 1980), though they may not be strongly attached to individual coral heads or sea anemones like anemonefishes (Fautin and Allen 1997). As adults, for example, the rabbitfishes may move over large reef areas as they forage (Popper and Gundermann 1975), and *P. miles*, while not attached to a single organism, rarely leave their home ranges within a reef (Fishelson 1975; Kochzius and Blohm 2005; Jud and Layman 2012). Sessile organisms that must rely on larval dispersal such as the coral, *Pocillopora verrucosa*, (Robitzch et al. 2015) and the sponge, *Stylissa carteri*, (Giles et al. 2015) also exhibit high levels of gene flow and admixture in the Red Sea. Thus, the level of gene flow within the northern Red Sea observed in these species and *A. bicinctus* is most likely due to the dispersal of pelagic larvae rather than adult movement.

Additionally, there are differences in reproductive methods and pelagic larval durations in these species compared to *A. bicinctus*. Anemonefish lay demersal eggs near the base of their host sea anemones that hatch into relatively well-developed larvae (Allen 1972; Fricke 1979; Fautin and Allen 1997) and have a 10 – 12 day pelagic larval duration (Nanninga et al. 2015). Similar to *A. bicinctus*, rabbitfishes lay demersal eggs, but the larval duration can last up to 45 days depending on the species (Duray 1998). *Pterois miles*, on the other hand, releases fertilized eggs in gelatinous masses that are positively buoyant (Fishelson 1975). While the pelagic larval duration is not known for *P. miles*, it has been estimated to last between 25 – 40 days (Hare and Whitfield 2003). Spawning behavior and larval duration have not been described for *L. quadrilineatus*, though many closely related labrids are broadcast spawners (Kuwamura 1981),
and the larval duration for *Labroides dimitiatus*, a close relative, has been estimated at 22 – 24 days (Brothers and Thresher 1985). Additionally, *L. quadrilineatus* larvae have a low estimated dispersal distance ranging from 0.44 – 5.1 km, yet populations, most likely through stepping-stone dispersal, experience high connectivity in the Red Sea (Froukh and Kochzius 2007). Thus, even with potentially smaller dispersal distances due shorter pelagic phases, *A. bicinctus* populations in the northern Red Sea (Nanninga et al. 2014; Saenz-Agudelo et al. 2015) and in the Gulf of Eilat experience enough gene flow, possibly through stepping-stone connectivity (Nanninga et al. 2014), to promote panmixia.

Despite the lack of genetic structure in our study, we observed low genetic diversity and high $F_{is}$ values. Such characteristics could potentially arise if a population relies heavily on self-recruitment rather than dispersal from other reefs, thus increasing the likelihood of inbreeding. We observed no self-recruitment in the present study despite ample recruitment having been observed in the Israeli site the previous year (Howell et al. 2016) The only parent assignments returned were from Cervus, and were only single parents at the relaxed confidence level, thus, for those Jordanian individuals that were assigned an Israeli parent, only a single member of the breeding pair (identified via population censusing, see Howell et al. 2016) was matched to an offspring. It is unlikely the single parent assignments were due to extra-pair mating events as anemonefish are monogamous (Fautin and Allen 1997) and the breeding pairs observed in Eilat tend to remain consistent over time (Howell et al. 2016). Additionally, those parent/offspring assignments were made when only Israeli breeding pairs were incorporated into the analysis. When potential Jordanian parents were included, no parent/offspring assignments were made. The lack of self-recruitment is consistent with the nearly nonexistent self-recruitment reported by Nanninga et al. (2015) for *A. bicinctus* in the main body of the Red Sea but is in contrast to
reports higher levels of self-recruitment in several other anemonefish species (Jones et al. 2005; Planes et al. 2009; van der Meer et al. 2012)

Even without self-recruitment, it might be expected, due to the prevailing counterclockwise current regime in the Gulf of Eilat, for *A. bicinctus* larvae to be dispersed from Jordan’s coastline around the tip of the Gulf to Israel’s reefs (Reiss and Hottinger 2012). The gyres that form seasonally in the Gulf of Eilat could also promote direct dispersal between the two sites (Berman et al. 2000). We did not observe any parent/offspring assignments that indicated direct dispersal between the sites at the strict confidence level or with positive LOD scores. Alternatively, since average counterclockwise current speeds within the Gulf of Eilat range from 5 cm s$^{-1}$ in summer to 20 cm s$^{-1}$ in winter ((Brenner et al. 1988; Genin 1994). *A. bicinctus* larvae dispersed from Jordan could potentially overshoot the Israeli site and settle farther down the coast. Also, via the same mechanism, *A. bicinctus* larvae that do settle in Israel may instead originate from reefs farther south on the Saudi Arabian coast, similar to dispersal pathways reported for *Chromis viridis* in the Gulf (Ben-Tzvi et al. 2008). Thus, cohorts of larvae may travel with the current north to Jordan and Israel where they settle. The very large estimated effective population sizes we calculated also support this, since a maximum of only 13 breeding pairs were observed over ~20 years of monitoring of the Israeli site (Howell et al. 2016),

Despite the lack of self-recruitment and genetic structure, the positive F$_{is}$ values, low genotypic diversity, and heterozygote deficiencies observed in our samples indicate elevated levels of inbreeding in this population of *A. bicinctus*, both within the Israeli and Jordanian sites and overall. Our observed values for F$_{is}$ were within the ranges reported by Saenz-Agudelo et al. (2015) for *A. bicinctus* throughout the entire Red Sea, but were larger than all but 2 of the 10 sites from which *A. bicinctus* were sampled. Additionally, the F$_{is}$ value we report for Jordan is
~200% larger than that reported by Saenz-Agudelo et al. (2015) farther south on the Saudi Arabian coast of the Gulf of Eilat. These differences could be due to gene flow from the Red Sea proper into the Gulf since the Saudia Arabian site is much closer to the Straits of Tiran than our northern Gulf sites. Additionally, differences in sample size (41 vs. 12), number of loci used (2001 vs. 4559), or when the samples were collected (1997/1998 vs. 2013; Saenz-Agudelo, personal communication) could also affect this parameter. Interestingly, such a decrease in the inbreeding coefficient over time could be indicative of larger breeding population sizes since lower $F_{is}$ values indicate the *A. bicinctus* population is gaining more genetic diversity.

Another potential cause of the low genetic diversity and inbreeding coefficient is a recent population bottleneck. BOTTLENECK (Cornuet and Luikart 1996), was unable to detect a recent population bottleneck in our samples. The negative values of Tajima’s $D$ and Fu’s $F_s$ and the non-significant results from the mismatch distribution and raggedness index are indicative of a population expansion. One possible cause of such an expansion could be the sea level decline during the last glacial maximum 15 – 20 thousand years ago. Water flow decreased between the Red Sea and the Gulf of Aden through the straits at Bab el Mandab, which was around 17 m and 6 km wide at its shallowest point, the Hannish sill (Werner and Lange 1975). The reduction in comparatively fresh water input from the Gulf of Aden and high levels of evaporation within the Red Sea resulted in much of the Red Sea becoming hyper saline and aplanktonic resulting in extirpation of many species (Fenton et al. 2000; reviewed in DiBattista et al. 2015). The northern Gulf of Eilat and southernmost portion of the Red Sea appear to have acted as refuges during the glacial maximum since they did not become aplanktonic according to sediment cores and presumably were within habitable salinity limits (Fenton et al. 2000). Thus, it is possible that *A. bicinctus* populations used the northern Gulf of Eilat as a refuge during the glacial maximum and
then expanded out into the rest of the northern Red Sea or re-colonized from populations in the southern Red Sea or Gulf of Aden. The latter is a more likely explanation for our results, considering water outflow from the Gulf of Eilat to the Red Sea occurs in deeper water layers in contrast to inflow of surface water, which may hinder larval transport out of the Gulf (Berman et al. 2000). Additionally, a previous population genetic study of *A. bicinctus* throughout the Red Sea proper and regions in the Gulf of Aden indicated unidirectional gene flow from south to north (Saenz-Agudelo et al. 2015), which corresponds to the general northward flow of water in the Red Sea (Sofianos and Johns 2003).

In light of the decline in abundance of both *A. bicinctus* and both of its host anemone species in the northern Gulf of Eilat (Howell et al. 2016), and a general decline or loss of species throughout the Gulf (Loya 1975; Fishelson 1995; Rinkevich 2005) the results presented here could have management implications for this species. Since *A. bicinctus* populations throughout the northern Red Sea experience enough gene flow to promote panmixia (Nanninga et al. 2014; Saenz-Agudelo et al. 2015), and do not appear to rely on self-recruitment for new individuals (Nanninga et al. 2015), other populations or locales could feasibly supply new recruits to the Israeli site at Eilat should the host anemone populations recover.
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LIST OF APPENDICES
Appendix A: Results from Average Nearest Neighbor analyses of sea anemone spatial distribution during 13 censuses (C1-C13) from October 1996 to August 1997. Spatial comparisons were run for *Entacmaea quadricolor* and *Heteractis crispa* combined (Total Anemones) and within each anemone species. The spatial distribution was either significantly clustered ($Z \leq -1.96$), significantly dispersed ($Z \geq 1.96$), or random ($-1.96 < Z < 1.96$).

<table>
<thead>
<tr>
<th>Census Number</th>
<th>Total Anemones</th>
<th><em>Entacmaea quadricolor</em></th>
<th><em>Heteractis crispa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z$</td>
<td>Distribution</td>
<td>$Z$</td>
</tr>
<tr>
<td>C1</td>
<td>-3.018</td>
<td>Clustered</td>
<td>-2.891</td>
</tr>
<tr>
<td>C2</td>
<td>-2.765</td>
<td>Clustered</td>
<td>-2.514</td>
</tr>
<tr>
<td>C3</td>
<td>-2.883</td>
<td>Clustered</td>
<td>-2.442</td>
</tr>
<tr>
<td>C4</td>
<td>-2.849</td>
<td>Clustered</td>
<td>-2.039</td>
</tr>
<tr>
<td>C5</td>
<td>-0.383</td>
<td>Random</td>
<td>-1.422</td>
</tr>
<tr>
<td>C6</td>
<td>-0.441</td>
<td>Random</td>
<td>-1.366</td>
</tr>
<tr>
<td>C7</td>
<td>-0.542</td>
<td>Random</td>
<td>-1.062</td>
</tr>
<tr>
<td>C8</td>
<td>-0.701</td>
<td>Random</td>
<td>-0.736</td>
</tr>
<tr>
<td>C9</td>
<td>-0.584</td>
<td>Random</td>
<td>-0.736</td>
</tr>
<tr>
<td>C10</td>
<td>-0.584</td>
<td>Random</td>
<td>-0.736</td>
</tr>
<tr>
<td>C11</td>
<td>-0.515</td>
<td>Random</td>
<td>-0.514</td>
</tr>
<tr>
<td>C12</td>
<td>-0.398</td>
<td>Random</td>
<td>-0.544</td>
</tr>
<tr>
<td>C13</td>
<td>-1.114</td>
<td>Random</td>
<td>-0.364</td>
</tr>
</tbody>
</table>
Appendix B: Results from Average Nearest Neighbor analyses of spatial distribution of uninhabited sea anemones during 13 censuses (C1-C13) from October 1996 to August 1997. Spatial comparisons were run for uninhabited *Entacmaea quadricolor* and *Heteractis crispa* combined (Total Anemones) and for uninhabited anemones within each species. The spatial distribution was either significantly clustered ($Z \leq -1.96$), significantly dispersed ($Z \geq 1.96$), or random ($-1.96 < Z < 1.96$).

<table>
<thead>
<tr>
<th>Census Number</th>
<th>Total Anemones</th>
<th>Entacmaea quadricolor</th>
<th>Heteractis crispa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z$</td>
<td>Distribution</td>
<td>$Z$</td>
</tr>
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<td>C1</td>
<td>-0.824</td>
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<td>-1.191</td>
</tr>
<tr>
<td>C2</td>
<td>0.232</td>
<td>Random</td>
<td>-0.775</td>
</tr>
<tr>
<td>C3</td>
<td>0.981</td>
<td>Random</td>
<td>0.790</td>
</tr>
<tr>
<td>C4</td>
<td>1.251</td>
<td>Random</td>
<td>-0.702</td>
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<tr>
<td>C5</td>
<td>1.100</td>
<td>Random</td>
<td>2.207</td>
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<td>1.765</td>
</tr>
<tr>
<td>C7</td>
<td>-0.738</td>
<td>Random</td>
<td>-0.731</td>
</tr>
<tr>
<td>C8</td>
<td>-0.240</td>
<td>Random</td>
<td>2.580</td>
</tr>
<tr>
<td>C9</td>
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<td>-1.586</td>
<td>Random</td>
<td>1.066</td>
</tr>
<tr>
<td>C11</td>
<td>-2.930</td>
<td>Clustered</td>
<td>0.135</td>
</tr>
<tr>
<td>C12</td>
<td>-1.803</td>
<td>Random</td>
<td>1.848</td>
</tr>
<tr>
<td>C13</td>
<td>-0.928</td>
<td>Random</td>
<td>0.627</td>
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</table>
Appendix C: Results from Average Nearest Neighbor analyses of spatial distribution of uninhabited sea anemones in relation to the nearest anemones inhabited by *Amphiprion bicinctus* during 13 censuses (C1-C13) from October 1996 to August 1997. Spatial comparisons were run for *Entacmaea quadricolor* and *Heteractis crispa* combined (Total Uninhabited vs. Total Inhabited). For each anemone species the spatial distributions of uninhabited anemones of that species in relation to any nearest anemone hosting an adult *A. bicinctus* were analyzed. The spatial distribution was either significantly clustered (Z ≤ -1.96), significantly dispersed (Z ≥ 1.96), or random (-1.96 < Z < 1.96).

<table>
<thead>
<tr>
<th>Census Number</th>
<th>Total Uninhabited vs. Total Inhabited</th>
<th>Uninhabited <em>Entacmaea quadricolor</em> vs. Anemones Hosting Adults</th>
<th>Uninhabited <em>Heteractis crispa</em> vs. Anemones Hosting Adults</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Z</td>
<td>Distribution</td>
<td>Z</td>
</tr>
<tr>
<td>C1</td>
<td>-5.406</td>
<td>Clustered</td>
<td>20.745</td>
</tr>
<tr>
<td>C2</td>
<td>-5.983</td>
<td>Clustered</td>
<td>-1.433</td>
</tr>
<tr>
<td>C3</td>
<td>-5.616</td>
<td>Clustered</td>
<td>0.050</td>
</tr>
<tr>
<td>C4</td>
<td>-6.304</td>
<td>Clustered</td>
<td>-2.281</td>
</tr>
<tr>
<td>C5</td>
<td>-5.785</td>
<td>Clustered</td>
<td>-3.298</td>
</tr>
<tr>
<td>C6</td>
<td>-5.632</td>
<td>Clustered</td>
<td>-4.507</td>
</tr>
<tr>
<td>C7</td>
<td>-5.198</td>
<td>Clustered</td>
<td>-4.053</td>
</tr>
<tr>
<td>C8</td>
<td>-5.811</td>
<td>Clustered</td>
<td>-4.001</td>
</tr>
<tr>
<td>C9</td>
<td>-5.900</td>
<td>Clustered</td>
<td>-4.411</td>
</tr>
<tr>
<td>C10</td>
<td>-5.929</td>
<td>Clustered</td>
<td>-4.532</td>
</tr>
<tr>
<td>C11</td>
<td>-5.086</td>
<td>Clustered</td>
<td>-4.442</td>
</tr>
<tr>
<td>C12</td>
<td>-4.710</td>
<td>Clustered</td>
<td>-4.219</td>
</tr>
<tr>
<td>C13</td>
<td>-4.412</td>
<td>Clustered</td>
<td>-5.661</td>
</tr>
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</table>
Appendix D: Results from Average Nearest Neighbor analyses of the spatial distribution of *Amphiprion bicinctus* during 13 censuses (C1 – C13) from October 1996 to August 1997. Spatial comparisons were run for the total anemonefish population (Total), the population divided into the three size classes (Adults, Juveniles, and Settlers), and Breeding Pairs. The spatial distribution was either significantly clustered ($Z \leq -1.96$), significantly dispersed ($Z \geq 1.96$), or random ($-1.96 < Z < 1.96$).

<table>
<thead>
<tr>
<th>Census Number</th>
<th>Total</th>
<th>Adults</th>
<th>Breeding Pairs</th>
<th>Juveniles</th>
<th>Settlers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z$</td>
<td>Distribution</td>
<td>$Z$</td>
<td>Distribution</td>
<td>$Z$</td>
</tr>
<tr>
<td>C1</td>
<td>-2.745</td>
<td>Clustered</td>
<td>-2.45</td>
<td>Clustered</td>
<td>0.44</td>
</tr>
<tr>
<td>C2</td>
<td>-2.361</td>
<td>Clustered</td>
<td>0.375</td>
<td>Random</td>
<td>1.421</td>
</tr>
<tr>
<td>C3</td>
<td>-3.164</td>
<td>Clustered</td>
<td>-0.382</td>
<td>Random</td>
<td>1.579</td>
</tr>
<tr>
<td>C4</td>
<td>-2.273</td>
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<td>-0.417</td>
<td>Random</td>
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</tr>
<tr>
<td>C5</td>
<td>-0.48</td>
<td>Random</td>
<td>0.566</td>
<td>Random</td>
<td>1.579</td>
</tr>
<tr>
<td>C6</td>
<td>-0.515</td>
<td>Random</td>
<td>0.999</td>
<td>Random</td>
<td>1.579</td>
</tr>
<tr>
<td>C7</td>
<td>-0.676</td>
<td>Random</td>
<td>0.465</td>
<td>Random</td>
<td>1.756</td>
</tr>
<tr>
<td>C8</td>
<td>-0.3</td>
<td>Random</td>
<td>1.372</td>
<td>Random</td>
<td>1.756</td>
</tr>
<tr>
<td>C9</td>
<td>-0.191</td>
<td>Random</td>
<td>1.669</td>
<td>Random</td>
<td>1.756</td>
</tr>
<tr>
<td>C10</td>
<td>0.171</td>
<td>Random</td>
<td>2.171</td>
<td>Dispersed</td>
<td>1.756</td>
</tr>
<tr>
<td>C11</td>
<td>-0.11</td>
<td>Random</td>
<td>1.857</td>
<td>Random</td>
<td>1.456</td>
</tr>
<tr>
<td>C12</td>
<td>-0.579</td>
<td>Random</td>
<td>1.476</td>
<td>Random</td>
<td>2.728</td>
</tr>
<tr>
<td>C13</td>
<td>-0.219</td>
<td>Random</td>
<td>1.234</td>
<td>Random</td>
<td>2.628</td>
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Appendix E: Results from Average Nearest Neighbor analyses of the spatial distribution of *Amphiprion bicinctus* size classes in relation to one another during 13 censuses (C1 – C13) from October 1996 to August 1997. Spatial comparisons were run for Settlers in relation to Juveniles, Adults, Juveniles + Adults, and Breeding Pairs. Spatial relationships were also determined for Juvenile anemonefish in relation to Adults. The spatial distribution was either significantly clustered ($Z \leq -1.96$), significantly dispersed ($Z \geq 1.96$), or random ($-1.96 < Z < 1.96$).

<table>
<thead>
<tr>
<th>Census Number</th>
<th>Settlers vs. Juveniles</th>
<th>Settlers vs. Adults</th>
<th>Settlers vs. Juveniles + Adults</th>
<th>Settlers vs. Breeding Pairs</th>
<th>Juveniles vs. Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z$</td>
<td>Distribution</td>
<td>$Z$</td>
<td>Distribution</td>
<td>$Z$</td>
</tr>
<tr>
<td>C1</td>
<td>6.133</td>
<td>Dispersed</td>
<td>64.838</td>
<td>Dispersed</td>
<td>3.624</td>
</tr>
<tr>
<td>C2</td>
<td>1.115</td>
<td>Random</td>
<td>16.736</td>
<td>Dispersed</td>
<td>-0.513</td>
</tr>
<tr>
<td>C3</td>
<td>0.317</td>
<td>Random</td>
<td>19.581</td>
<td>Dispersed</td>
<td>-1.434</td>
</tr>
<tr>
<td>C4</td>
<td>1.704</td>
<td>Random</td>
<td>19.817</td>
<td>Dispersed</td>
<td>-0.591</td>
</tr>
<tr>
<td>C5</td>
<td>1.244</td>
<td>Random</td>
<td>19.697</td>
<td>Dispersed</td>
<td>-0.214</td>
</tr>
<tr>
<td>C6</td>
<td>0.001</td>
<td>Random</td>
<td>11.789</td>
<td>Dispersed</td>
<td>-1.409</td>
</tr>
<tr>
<td>C7</td>
<td>-0.454</td>
<td>Random</td>
<td>11.31</td>
<td>Dispersed</td>
<td>-2.342</td>
</tr>
<tr>
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<td>-1.494</td>
<td>Random</td>
<td>8.571</td>
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</tr>
<tr>
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<td>-0.417</td>
<td>Random</td>
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</tr>
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<td>C10</td>
<td>-0.75</td>
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<td>6.5</td>
<td>Dispersed</td>
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</tr>
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<td>C11</td>
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<td>Random</td>
<td>5.336</td>
<td>Dispersed</td>
<td>-3.304</td>
</tr>
<tr>
<td>C12</td>
<td>-2.183</td>
<td>Clustered</td>
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<td>Dispersed</td>
<td>-4.07</td>
</tr>
<tr>
<td>C13</td>
<td>-2.905</td>
<td>Clustered</td>
<td>0.347</td>
<td>Random</td>
<td>-5.687</td>
</tr>
</tbody>
</table>
VITA

Jacob Howell
Biology Department
526 Shoemaker Hall
University of Mississippi
University, MS 38677
(256) 478-6244
jhowell3@go.olemiss.edu

Education:

University of Mississippi, University, MS
• GPA: 4.0
• Master of Science in Biological Sciences
  o Advisor: Dr. Tamar L. Goulet
  o June 2016

University of South Alabama, Mobile, AL
• GPA: 3.87
• Bachelor of Science in Biological Sciences with Honors
  o Concentration in Marine Biology
  o Minor in Chemistry
  o Magna Cum Laude
  o May 2013
• GRE Scores:
  o Verbal – 160
  o Quantitative – 156
  o Analytical Writing – 4.5

Teaching/Work Experience:

2015 – 2016 Lafayette County Schools – Science Fair Judge
• Interviewed local school students about their science fair projects
• Assigned points to each project based on thoroughness, difficulty, originality, and the student’s knowledge and presentation abilities

2014 – 2015 3-Minute Thesis Competition
• Condensed master’s thesis research into a 3-minute oral presentation for a lay audience
• Chosen to move on to semi-finals (2015)

2013 – Present University of Mississippi – Graduate Teaching Assistant
• Courses Taught: BISC 103, BISC 105, BISC 161, BISC 163
• Taught laboratory courses for the introductory biology courses for both non-science majors and science majors
• Responsible for grading quizzes, worksheets, presentations, and reports
• For BISC 163, responsible for writing weekly quizzes
• Proficient at working with and organizing course material on BlackBoard

2012 – 2013 Science Olympiad - Volunteer
• Helped prepare kits and materials for the “Write It / Do It” event
• Served as scorer and helped run the “Write It / Do It” event during the 2012 Science Olympiad competition at Springhill College in Mobile, AL

2011 – 2013 The Vanguard – The University of South Alabama’s Student Newspaper
• Summer ‘12 – Spring ’13 – Section Editor
• Fall ’11 – Spring ’12 – Contributing Writer
• Wrote newspaper articles for the Jaglife section of The Vanguard
• As Editor, managed and assigned article topics to freelance writers
• Proof-read and edited articles for the JagLife section
• Built and designed the layout of the JagLife section for each week’s edition of The Vanguard
• Conducted interviews and produced photographs to accompany articles

2011 Natural Resources Conservation Service – Farm City Event
• Volunteered during Farm City, an event aimed at educating local grade school children about farming and agricultural practices as well as where their food comes from
• Painted mural for Farm City Event
• Volunteered during set-up for Farm City Event

2011 – 2013 University of South Alabama Supplemental Instruction (SI) Leader
• Attended general biology lectures multiple times per week
• Provided additional instruction and aid for general biology students outside of class multiple times per week
• Worked with instructors of record to provide input on exam questions
• SI leader for BLY 101 (Life Sciences, non-science majors) and BLY 121 (General Biology, science majors)

2010 – 2012 Institute of Marine Mammal Studies
• Volunteer Experience
• Aided IMMS staff in public educational programs
• Served in the IMMS museum, guiding visitors through exhibits and activities
• Assisted IMMS staff in touch pool presentations
• Aided IMMS staff in animal care (food preparation, feeding, water quality tests, medication preparation, etc) for bottlenose dolphins, rescued/rehabilitated sea turtles (4 species), and various organisms housed in aquaria and the touch pool
• Volunteer for the Mississippi and Alabama Dolphin Stranding Networks

2006 – 2009 Natural Resources Conservation Service - Etowah County Water Festival
• Lead and taught workshops and activities instructing local fourth grade classes about the water cycle – Edible Aquifers
Publications and Presentations:


2013  Howell, J., Undergraduate Honors Thesis: The effects of crude oil exposure on molt cycle duration in the grass shrimp, *Palaemonetes pugio*, and the blue crab, *Callinectes sapidus*; Mentor: Dr. John Freeman, University of South Alabama


Research Experience:

2014 – Present  Analysis of spatial patterns of settlement, habitat usage, and survival of a population of *Amphiprion bicinctus* in the Gulf of Aqaba, Israel over time using tools such as ArcMAP and R, Master’s Thesis Research

Determining if a population of *Amphiprion bicinctus* in the Gulf of Aqaba, Israel self-seeds through the use of triple-digest restriction-site associated DNA (3RAD) sequencing, Master’s Thesis Research

2014  Assisted on a polyp activity survey of Caribbean gorgonians and collected tentacle samples from *Condylactis gigantea* sea anemones in Puerto Morelos, Mexico, Field Research Experience
2013  Effects of two endocrine disruptors on repetitive DNA element expression in the zebrafish, *Danio rerio*, Animal Physiology Independent Research Project

2011 – 2013  Determining if exposure to crude oil affects the molt cycle duration in grass shrimp, *Palaemonetes pugio*, and the blue crab, *Callinectes sapidus*, collected from Meaher State Park, AL through the use of microcosm experiments, Undergraduate Honors Thesis Research

**Grants and Fellowships:**

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<tr>
<td>2014 – 2015</td>
<td>University of Mississippi Graduate Student Council: Graduate Student Research Grant, $1,000</td>
</tr>
<tr>
<td>2014 – 2015</td>
<td>Sigma Xi (G20121015161768): Grants-in-Aid of Research, $987</td>
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<td>2014</td>
<td>McRight Fellowship - $2,000</td>
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**Academic Honors:**

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<td>2013</td>
<td>Society of Professional Journalism Mark of Excellence Award (2nd Place – Column Writing)</td>
</tr>
<tr>
<td>2012</td>
<td>John M. Rawls Award in Biology (Annual award given to an outstanding senior biology major at the University of South Alabama)</td>
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<td>President’s List</td>
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<tr>
<td>2011</td>
<td>Mortar Board Honor Society</td>
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<td><em>Phi Kappa Phi</em> Honors Society</td>
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<td>2011</td>
<td>University of South Alabama Endowed Biology Scholarship</td>
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<td>Outstanding First Year Spanish Student</td>
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<tr>
<td></td>
<td>President’s List</td>
</tr>
<tr>
<td>2010</td>
<td>President’s List</td>
</tr>
<tr>
<td></td>
<td>National Society of Collegiate Scholars</td>
</tr>
<tr>
<td>2009</td>
<td><em>Phi Eta Sigma</em> Honor Society</td>
</tr>
<tr>
<td></td>
<td>Dean’s Honor List</td>
</tr>
<tr>
<td></td>
<td>Frederick P. Whidden Honors Scholar</td>
</tr>
</tbody>
</table>

**Organizational Membership and Involvement:**

<table>
<thead>
<tr>
<th>Year</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016 – Present</td>
<td>International Society for Reef Studies (Student Member)</td>
</tr>
<tr>
<td>2014 – Present</td>
<td>Divers Alert Network</td>
</tr>
</tbody>
</table>
2012 – 2013 Mortar Board Honor Society
• Office held: Historian
  o Attended and chronicled all Mortar Board events using photography
2009 – 2013 University of South Alabama Honors Program Organization
• Office held: President (Fall ’12 – Spring ’13)
  o Presided over all Honors Program meetings
  o Organized Honors Program Study Abroad scholarship program
  o Aided in organization and execution of Ghouls for Good canned food drive for the Bay Area Food Bank
  o Organized Honors Program involvement in Relay For Life
  o Represented the Honors Program at the USA Board of Trustees Meeting
• Office held: Fundraising Coordinator (Fall ’10 – Spring ’11)
  o Aided in the planning and execution of various fundraising and social projects for the Honors Program
• Other activities
  o Honors Program Class Whip
  o Honors Program Student Council
2009 – 2013 University of South Alabama Biology Student Association
• Office held: Vice-President (Fall ’11 – Spring ’13)
  o Aided in organization of speaker series for bi-monthly meetings
  o Participated as judge for Science Olympiad event – Write-it/Do-it
  o Participated in Coastal Clean-up events
  o Organized various fundraising events
  o Participated in Relay For Life and Oozeball charity events

Certifications:
2014 – Present American Heart Association
• Heartsaver® CPR/AED/First Aid
2014 – 2015 American Academy of Underwater Sciences
• Scientific Diver
2013 – Present Scuba Schools International
• Open Water Diver
• Advanced Open Water Diver
• Logged Dives: 30