The expansion and impact on native species of a sea anemone introduced into a tropical marine lake (Jellyfish Lake, Palau)

A Thesis submitted in partial satisfaction of the requirements for the degree of Master of Science in Quantitative and Systems Biology by Sharon Patris

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ABSTRACT

Introduced species often are considered one of the major threats to biodiversity in island and marine habitats. However, generalizations from anecdotal observations, bias in scientific literature, and the unpredictability of species’ responses to new environments have limited our understanding of the impact of invasive species. To address this limitation, there is a need for an increase in quantitative studies that objectively assess occurrence, distribution and impact of non–native species in systems that are under–represented in the scientific literature. Here, we describe the introduction of a non-native sea anemone species into Jellyfish Lake, an isolated tropical marine lake. From 2003–2012, we gathered samples to identify the introduced species and quantitative data using transect and photo–quadrat surveys to describe its abundance and distribution. We also assessed its impact on native species through possible competition for space. The non–native species was found at the tourist entry into the lake in 2003 and identified as *Exaiptasia* sp., a symbiotic sea anemone; temporal patterns of tourism, the spatial extent of the anemone, and genetic analyses of the symbiont, were consistent with the early stages of invasion. Subsequent expansion of *Exaiptasia* sp. occurred within six years, fully encompassing the perimeter of the lake, occurring predominately on mangrove roots. Species' assemblages that experienced invasion were significantly different among surveys and habitats. Using Pearson’s and Spearman rank correlation, we determined both positive and negative relationships between the percent cover of *Exaiptasia* sp. and a subset of 20 native species that were present in all four surveys. Overall, *Exaiptasia* sp. percent cover had a significant but weak negative correlation (-0.5 < r < -0.3) with diversity only on mangrove roots only in March and August 2009. The rapid expansion of *Exaiptasia* sp. but the lack of strong effect raises a quandary for management: to act or not act to prevent introductions into Jellyfish Lake and other marine lakes.

Keywords


CHAPTER 1: MANUSCRIPT

INTRODUCTION

Detrimental impacts of non–indigenous species (NIS) on native species, ecosystem functions, and society often have been highlighted in descriptions of species invasions (Bax et al. 2003; Galil 2007; Molnar et al. 2008) leading, in some cases, to concerns that impacts may have been over–emphasized (Davis 2003; Sax & Gaines 2003). The over–generalization that introductions lead to invasions with detrimental effects, and that the abundance of NIS correlates with the magnitude of impact, may impede our understanding of introductions (Gurevitch & Padilla 2004; Ricciardi & Cohen 2007). Moreover, misunderstanding of invasions subsequently may detrimentally affect efforts to prevent and manage introduced species (Forrest & Taylor 2002; Ricciardi & Cohen 2007). Studies that measure the occurrence, distribution and assess the impact of novel NIS in well delineated systems and lead to commensurate management efforts may thus be important for understanding the scope and scale of impacts of NIS.

Multiple processes and factors can influence relationships between native and exotic species richness, leading to both positive and negative correlations between numbers of NIS and endemics in fine–scale studies covering areas of <10 hectares (see Sax & Gaines 2003; Fridley et al. 2007). For example, the trophic position of NIS and whether they are predators, pathogens or competitors can influence impacts on native species. Introduced predators and pathogens are more likely to cause severe reduction in native abundances and subsequent extinctions than are introduced competitors (Sax & Gaines 2003; Thomsen et al. 2014a); there is little evidence of NIS plants outcompeting native plants for space, suggesting that certain introductions can increase biodiversity (Sax & Gaines 2003; Thomas & Palmer 2015). Abiotic and biotic characteristics of the recipient ecosystem also must be taken into account in predicting the invasive potential and possible impact of NIS (Fridley et al. 2007; Schaffelke & Hewitt 2007; Ricciardi et al. 2013). These characteristics include habitat alteration and human exploitation; disturbed areas are more susceptible to invasions (Vitousek et al. 2007). This means loss of biodiversity often may have been attributable to confounding factors of NIS, habitat alteration, and human exploitation (Davis 2003; Sax & Gaines 2003; Gurevitch & Padilla 2004; Vitousek et al. 2007) and not necessarily indicative of generalized effects of NIS alone.

In addition, for a number of historical reasons, literature is biased towards temperate regions, with few studies covering tropical or polar regions (Molnar et al. 2008; Fridley et al. 2007; Thomsen et al. 2014b). A majority of studies also cover terrestrial and freshwater habitats, with less research focusing on marine habitats such as sandy bottoms and coral reefs (Thomsen et al. 2014b). Sessile organisms, such as molluscs, algae and ascidians are widely reported in invasion literature, while non–native marine fishes and cnidarians are under–represented (Molnar et al. 2008; Thomsen et al. 2014b). There is also a lack of available long–term data, both present and historic, on the abundance of native species (Sax & Gaines 2003).

These biases exacerbate existing difficulties of studying NIS, especially in marine systems. Limitations of traditional morphological taxonomy and a dearth of historical
data have made it difficult to correctly identify species as native or introduced, leaving ~30–40% of marine taxa as cryptogenic (Carlton 1996; Hewitt et al. 2004; Eldredge & Carlton 2015). In addition, presumably high connectivity—due to few clearly defined boundaries and oftentimes high dispersal potential of marine organisms—make it difficult to document and predict the expansion of introduced marine species (Holland 2000; Dawson et al. 2005). The use of genetic analysis may resolve some of these issues in studying NIS introductions (Holland 2000; Dawson et al. 2005); however, logistical issues remain in conducting studies in large scale, poorly delineated systems with cryptic taxa.

These problems are greatly reduced in marine lakes—isolated bodies of seawater entirely surrounded by land (Dawson & Hamner 2005). Marine lakes offer an opportunity to study the ecology and evolution of NIS in marine environments in situations that are analogous to terrestrial islands (Dawson 2015). In marine lakes, as on terrestrial islands, colonization is dependent on the degree of isolation, size (presumably as a proxy for habitat and resource availability), age, and traits of colonists (Dawson et al., in press). Marine lakes may represent processes in other island–like marine environments (Dawson 2015; Hachich et al. 2015) and in other situations in which invasions have often been studied, such as semi–enclosed bays, estuaries and seas. However, marine lakes have an advantage over these areas that are open to heavy ship traffic and human development and that have experienced waves of introduced species over the last century (Galil 2007). Marine lakes are largely new to exploration and exploitation, and these relatively pristine ecosystems provide an opportunity to study marine introductions in clearly defined areas where abiotic conditions and biological communities can be easily monitored. Marine lakes may provide a model system to study how newly introduced species respond to a new environment and how resident species respond to a new addition in their environment.

The primary goal of this study is to describe the distribution and spread of an introduced species of sea anemone in a tropical marine lake, called Jellyfish Lake, Palau, since 2003. We also seek to determine the current impact of the introduced anemone on native species through possible competition for space.

METHODS

Study site

Jellyfish Lake, or Ongeim'l Tketau (OTM), is one of 57 marine lakes in Palau (Colin 2009). OTM has an area of 61,000 m² and its deepest depth is 30 m (Colin 2009). It is a closed and isolated lake completely surrounded by karst island. The lake’s tide is damped to 0.80 m, less than half of the lagoon’s 2 m tidal range, and delayed by ~2.5 hours (Hamner & Hamner 1998). The damped and delayed tide is indicative of the isolation of the lake; the lake retains an indirect and limited connection to the surrounding ocean through small tunnels, cracks and crevices. As a consequence, the lake is stratified and sensitive to changes in climate, as evidenced by the correlation of the lake’s abiotic environment with ENSO conditions (Martin et al. 2005) and a jellyfish population crash following the extreme 1997–1998 El Niño–La Niña (Dawson et al. 2001).
OTM has ≥59 benthic species and 4 fishes (L. Bell, pers. comm.), which are restricted to the upper oxygenated 15m deep epilimnion. Though not as species rich as reef communities, OTM has a high proportion of endemic populations and species (Hamner & Hamner 1998; e.g. Fautin & Fitt 1991; Dawson & Hamner 2005).

Formed 10,000–15,000 years ago, OTM has been isolated from human contact for most of its history. OTM does not harbor large fish, and it was not a traditional fishing ground nor was it used for fish grow–out as evidenced in other lakes scattered around the rock islands. However, OTM became a tourist attraction for international visitors in 1986 because of its unique endemic population of *Mastigias* jellyfish (Etpison 1997). By 1994, there were approximately 2,500 visitors per month (Hamner 1994), which rose rapidly to ~6,800 visitors per month by 2011 and now stands at ~11,000 visitors per month in 2014 (Koror State Visitor's 2011–2014 Logbook, unpublished). Thus, due to its unique biological diversity, OTM has become an important economic resource—garnering millions of dollars annually from the heavily marketed tourist industry, one of Palau’s main income resources. At the same time, the increase of visitor traffic has opened OTM to an elevated possibility of introduction of non–native species. Reef shells, a fungiid coral, and a sand dollar have been found near the entry dock, carried over by visiting groups (L. Bell, M.N Dawson pers. comm.).

**Discovery of introduction and identification, November 2003**

We have been monitoring OTM monthly since December 1998, documenting physical parameters of the lake (i.e. temperature, salinity, oxygen, and pH) and population sizes of plankton. During these monthly visits, we also visually inspected areas around the lake and recorded qualitative observations of the lake’s perimeter, especially in a side–arm of the lake where a floating dock facilitates visitors’ entry into the lake. On 14 November 2003, a patch of sea anemones—morphologically distinct from the native sea anemone *Entacmea medusivora*—was discovered in the side–arm in front of the entry dock. Specimens were collected from this patch, and from other locations in subsequent years, preserved in 10% buffered formalin in seawater or 95% ethanol, and sent to specialist taxonomists for identification and DNA sequencing. During preliminary investigations, it became apparent that the anemone was symbiotic with zooxanthellae, and we sent samples of the dinoflagellate for DNA sequencing too.

**Quantitative Surveys, 2003 to 2008: Population expansion**

On 22 November 2003 we began quantitative surveys of the novel sea anemone in the side–arm of the lake. We documented the occurrence of introduced sea anemones along eight parallel transects, each six meters apart. Transects ran on an approximately east–west axis across the side–arm of the lake, with a search boundary of 50 cm to the left of the transect tape. Whenever we encountered a sea anemone in this swathe, we placed a 50 cm x 50 cm quadrat alongside the transect tape with the sea anemone placed inside the bottom left corner of the quadrat. We enumerated the number of 10 x 10 cm squares that had at least one sea anemone. If patch area was larger than 50 cm², the quadrat was flipped along the transect line and additional cells were counted until no sea anemones were seen within 10 cm of the edge of the quadrat. Depth, substrate and distance along transect were recorded. Transects were repeated on 9 November 2004, 30 September 2005, and 01 December 2006. The original method was modified slightly in 2004–2006 due to a substantial increase in coverage of the sea
anemone; for these transects, the quadrat was placed every two meters from 0 m to the end of each transect.

In addition, on all four dates, we visually inspected areas adjacent to the side–arm and noted patches of non–native sea anemone found outside the side–arm, in the main basins of the lake. We expanded the survey outside the side–arm in 2006. The extended surveys in 2006 and 2007 were conducted using a Global Positioning System (GPS; model Garmin 60CSx) to mark locations of patches beyond the side–arm and past the invasion front, i.e. the furthest edge of the continuous line of the introduced anemone. The continuous line was defined as patches of the introduced sea anemone being separated by no more than 10 m distance.

**Quantitative Surveys, 2009 to 2012: Occurrence and possible impacts**

We conducted two types of surveys in March 2009, August 2009, August 2010 and August 2012: (1) transect surveys and (2) photo–quadrat surveys. All surveys were conducted through snorkeling and circumscribed the entire lake.

**Transect survey: density, distribution and extent of the non–native anemone**

The transect surveys documented density, distribution and extent of the non–native sea anemone along the perimeter of lake. Using a transect tape laid along the shallow edge of the lake, we recorded non–native sea anemone patch density at 10 m intervals. At each 10 m interval, we searched for non–native sea anemones in a ~5 sq. meter area, and scored each area based on the percent cover of the sea anemone. Patch sizes were rated on a 0–5 point scale representing 20% intervals of cover, i.e. 0 for no introduced sea anemones, 1 for 20% cover, 2 for 40% cover, and so on. Each sample location was marked with a GPS position; however, high karst ridges and overhanging trees limited accuracy of marked waypoints. Waypoints were mapped onto a Google Earth aerial image of OTM, and inaccurate points (e.g. farther than 10m from the perimeter) were re–mapped onto the shoreline of the lake between the immediately preceding and following data points.

**Photo–quadrat survey: abundance and impact of the non–native anemone**

The photo–quadrat surveys were conducted to study the abundance of non–native sea anemone and endemic species in the lake and determine relationships between their abundances. Quadrats were placed at 60 random sites around the circumference of the lake; distances between sites ranged from 2–80 m. We repeated the survey, each time with new random positions, for a total of 4 times. During each survey, a transect tape was laid along the perimeter of the lake at 1–3 m depth. A 0.5 m² quadrat was placed 0.5 m below the mid–tide mark, which is indicated by a line of mussels. Photographs were taken with the 0.5 m² quadrat filling the camera frame. To increase resolution and ease identification, we also took additional photographs of each half quadrat, quarter quadrat, and close up photographs of individual species therein, using a flash as necessary. In places where the quadrat could not be placed down (e.g. because of a sheer drop–off), we moved the site one meter to the left or to the closest area where it was possible to place the quadrat.

Percent cover of species in the lake was calculated using the random point count methodology through the Coral Point Count with Excel extensions (CPCe; Kohler & Gill
2006). Using 100 random points placed across each quadrat photo, species on which crosshairs fell were identified against a list of species in OTM. Where necessary, we used close up photos of quadrat cells to identify species. Sponges that were difficult to visually distinguish with accuracy, such as small encrusting yellow sponges, were lumped into color and morphological categories: encrusting, palmate, or possessing tendrils.

To test for differences in assemblages among surveys, data were square root transformed to de-emphasize common species and allow intermediate species to contribute to the Bray–Curtis similarity matrix (Clarke & Warwick 2001). To visualize how community composition may vary based on factors of habitat and time, we ran nMDS and PCO ordination plots using Primer–E v.7 (Clarke & Gorley 2015). Habitat was categorized into mangrove roots and other near-surface substrate (ONS); ONS encompasses all other substrate, such as mud, rock, wood, algae, and broken shells. ONS sites were mostly a mixture of mud and rock, with a smaller fraction of wood, broken shells and algae. As sites were separated by habitat, we ran permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to test differences among surveys within habitats using 999 permutations. In addition, we ran permutational test of multivariate dispersion (PERMDISP; Anderson 2006) to test for homogeneity of multivariate dispersions. SIMPER (Clarke 1993) was used to test which species contributed the most to the similarity matrix within groups and the dissimilarity between groups.

To determine composition of benthic assemblages, frequency of presence of species found in quadrats was calculated per survey and compared to mean percent cover per survey. Finding that frequency in quadrats relates to mean percent cover per survey, species were grouped based on their frequency in the total number quadrats across all surveys. Species were categorized as constant (present in all four surveys), and predominant (occurs in ≥50% of quadrats), common (occurs in 20–50% of quadrats), or rare (occurs in <20% of quadrats). Because categories were based on presence and frequency, species were grouped under the combination of the category “constant” and any of the other three categories: “predominant,” “common,” or “rare.”

To understand factors determining native diversity and non-native abundance, including relationships between the two, we first ran an ANOVA for (i) Shannon–Weaver Index calculated for all native species in each quadrat and (ii) the percent cover of introduced anemone. ANOVA is robust to deviations from normality if the assumption of homogeneous variance is not violated, which we tested using Cochran C–Test. Data was transformed when necessary to remove heteroscedasticity, and in this case, non-native sea anemone percent cover was log(X + 1) transformed. Survey dates were set as a fixed factor of 4 levels (March 2009, August 2009, August 2010, and August 2012). Habitat was set as a fixed factor of 2 levels (Mangrove, ONS). When converting to number of effective species (i.e. exp(H)), results of ANOVA and Cochran C–test were similar to those for the Shannon–Weaver Index.

Finally, we used Pearson’s correlation and Spearman rank correlation to describe relationships between the non-native sea anemone and (a) overall native diversity and (b) the constant species for each survey within each habitat. Pearson’s correlation, a parametric test with stronger power than non-parametric tests, assumes normality, but is still robust to violations of this assumption. For comparison of results,
and because sample sizes are ≤40 with zero–value inflations, we also use the non–parametric test of Spearman rank correlation to identify potential significant relationships under lower power (0.5–0.7). Power analysis was conducted post–hoc to identify correlations with 0.5–0.8 power, using sample sizes for each group, calculated r coefficients, alpha or significance level of 0.05.

RESULTS

Discovery of introduction and identification, November 2003

The first discovery of the non–native sea anemone was of a ~3 m² patch at ~3 m depth immediately in front of the visitors' entry dock on November 14, 2003. Specimens were identified as Exaiptasia sp. (Aiptasiidae) through morphological taxonomy (M. Daly, A. Grajales pers. comm.), and its zooxanthellae were identified through DNA sequencing. Exaiptasia sp. samples, collected in 2003, harbored Symbiodinium clade D (n = 3). In 2005, Exaiptasia sp. samples harbored Symbiodinium clade D (n = 18) or a mixture of clades B and D (n = 1). Exaiptasia sp. in 2006 harbored Symbiodinium clade D (n = 24), or clade B (n = 5), or clade C (n = 5), or a combination of clades D and C (n = 1). Zooxanthellae from the Exaiptasia sp. samples collected in 2015 (n = 5) were identified as Symbiodinium minutum (formerly type B1; T. LaJeunesse, pers. comm.).

Quantitative Surveys, 2003 to 2008: Population expansion

Over a period of three years, Exaiptasia sp. spread rapidly, covering mangrove and rock substrate in the side–arm where the tourist dock is located (Figure 1). The substrate in the side–arm is mostly rock and mangrove roots in the intertidal, with a subtidal rocky slope that gives way at 6 m depth to a flat bottom of flocculent mud. There were no Exaiptasia sp. patches on the flocculent mud at the bottom. By 2006, 76% of quadrats on mangrove roots, rock and/or algae substrates within the side–arm had >80% cover of Exaiptasia sp.

Exaiptasia sp. patches were first observed outside the initial survey zone in 2004, along the northern perimeter of the western basin of the lake (Figure 2A). A year later in 2005, patches of Exaiptasia sp. were found along the western and northern rim, in areas adjacent to the side–arm. By 2006, the introduced Exaiptasia sp. population formed a continuous line more than 100 m outside the initial invasion area along both the northern and western perimeter. A few satellite patches extended past the continuous line in the west basin, and only one small satellite patch was found in the east basin. In 2007, Exaiptasia sp. had spread fully around the west basin in a continuous line with many satellite patches in the east basin; 70% of the satellite patches in the east basin were <1 m².

Quantitative Surveys, 2009 to 2012: Occurrence and possible impacts

Transect survey: density, distribution and extent of the non–native anemone

In March 2009, Exaiptasia sp. had spread throughout the east and west basins of the lake, covering mangrove roots and hard substrate along the sides of the lake (Figure 2B). Areas with no Exaiptasia sp. occurred at a frequency of 1–3% for the first three
surveys, but jumped to 40% in 2012. Over half of the sites had 20% Exaiptasia sp. cover per survey. The number of areas with 40% and 60% cover was variable among surveys but showed a decreasing trend from March 2009 to August 2012. Areas with scores of 80% and 100% were the least common categories of sites, and further decreased to a frequency of only 1% in August 2012.

**Photo–quadrat survey: abundance and impact of the non–native anemone**

Results indicate that assemblage of species (i.e. percent cover or abundance) in OTM change through time, and a different composition of species were found on mangrove roots and ONS habitats. Assemblage composition differed significantly according to habitat (Pseudo F (F) = 10.88, Perm p–value (p) = 0.001, PERMPDISP P (P) = 0.001) and survey dates (F = 5.85, p = 0.001, P = 0.037), including a significant interaction between habitat and survey dates (F = 2.18, p = 0.007). Moreover, mangrove habitat assemblages showed significant differences among surveys (F = 2.64, p = 0.005, P = 0.01). ONS habitat showed significant differences among surveys (F = 5.70, p = 0.001). However, PERMDISP values for ONS habitat were not significant (P = 0.17). Significant PERMANOVA F indicated that centroids of groups differ in location, and significant PERMDISP indicated that the difference in location is driven by differences in variance for each group. The significant PERMANOVA and PERMDISP values indicated that the difference between assemblages according to factors of habitat and survey dates is influenced by the differences in variance within the groups. A non–significant PERMDISP for ONS habitat indicated that differences in variances within groups (of survey dates) were not driving differences between centroid locations. ONS species’ percent cover were similar among the survey dates, whereas species' percent cover on mangrove roots were variable.

SIMPER results indicated that, overall, Caulerpa fastigiata, Caulerpa verticillata, Brachidontes sp. 2, and Exaiptasia sp. were the top four species driving similarity averages within surveys and dissimilarity averages among surveys. In 2012, however, Exaiptasia sp. contribution was its lowest (~3.5%) of all four surveys, and the 'encrusting sponge' category and Polycarpa tumida (an ascidian) were listed along with C. fastigiata, C. verticillata, and Brachidontes sp. 2 as major contributors to the similarity between quadrats.

Of the 59 known benthic species in Jellyfish Lake, 45 species were present and identified in photo–quadrats, of which only 20 species were present in all four surveys. Four species were predominant (i.e. occurred in ≥50% of total quadrats): the influential two macroalgae (C. fastigiata, C. verticillata), mussel (Brachidontes sp.2), and the non–native anemone (Exaiptasia sp.). Ten additional species were common (i.e. occurred in 20–50% of quadrats): an ascidian (P. tumida), a gastropod (Drupella margaritcola), seven sponges (Cinachyrella australiensis, Dendrilla lendenfeldi, Dragmacidon sp. 1, Haliclona sp. 16, Suberites diversicolor, Suberea sp. 2, Tethya sp. 2, and the generic encrusting sponge), and cyanobacteria. All other species were identified as rare (i.e. in ≤20% of quadrats), including six that occurred in all four surveys: the native sea anemone (Entacmaea medusivora) and five sponges (Dendrilla sp. 3, Haliclona sp. 13, Hymeniacidon sp. 1, Tethya microstella, and Spheciospongia peleia).

The Shannon–Weaver index for native species ranged from 0 to 2.5. Diversity varied between quadrats among survey dates and between habitats (Figure 3B). Median
Exaiptasia showed consistent negative relationships with mangrove root and C. verticillata August 2009 and August 2012. Exaiptasia with occurred in surveys, when August 2009, Almost all significant correlations were found within the first three surveys (March and common native species occurred principal correlation. Spearman rank correlation identified more significant correlations between Exaiptasia sp. percent cover of Exaiptasia sp. per survey was highest (15%) in March 2009 (Figure 3A) and lowest in August 2012 (3%). Higher cover of Exaiptasia sp. occurs on mangrove roots than ONS (Figure 3A), with the highest mean percent cover on mangrove roots found in March 2009 (23% mean cover) and the lowest mean percent cover in August 2012 (4% mean cover). ONS substrate averaged 3% percent cover of Exaiptasia sp. for all surveys with the highest percent cover in March 2009 (6% cover) and lowest in August 2012 (0.5% cover).

Using Pearson’s correlation, we identified 14 significant correlations between the percent cover of Exaiptasia sp. and a subset of “constant” native species. Constant native species are species present in all four surveys. In ONS habitat, there were four positive significant correlations (Table 1). There were 10 significant correlations on mangrove roots, of which three were positive relationships. A majority of significant correlations occurred in the first 3 surveys; August 2012 survey had only 2 significant relationships. No significant correlations were found between Shannon–Weaver Index and Exaiptasia sp. percent cover. Sample sizes per habitat within survey date ranged from 22–38. There was an even distribution of habitats in March 2009 and August 2009, whereas in August 2010 the ONS sites numbered 22 and mangrove root sites were 38. In August 2012 there were 25 ONS sites and 35 mangrove roots sites. The variable sample sizes of each group affected power for each correlation, but generally, correlations of r ≥ 0.4 had power ≥ 0.8. Only a few correlations (n=14) of nine native species and Exaiptasia sp. percent cover achieved this r value.

Spearman rank correlation identified more significant correlations between Exaiptasia sp. and the predominant and common native species than did Pearson’s correlation. Significant correlations between Exaiptasia sp. and the predominant and common native species occurred principally in the mangrove root habitat (Table 2). Almost all significant correlations were found within the first three surveys (March and August 2009, August 2010). There were only four significant correlations in August 2012, when Exaiptasia sp. was at its lowest abundance. Of the twenty species that consistently occurred in surveys, percent cover of fourteen species showed significant correlations with Exaiptasia sp. percent cover. Considering the three predominant native species, Exaiptasia sp. and Brachidontes sp. 2 had negative relationships on mangrove roots in August 2009 and August 2012, while Exaiptasia sp. was negatively correlated with Caulerpa fastigiata on mangrove roots in March 2009 and August 2010. Exaiptasia sp. and C. verticillata were positively correlated on ONS (March 2009 and August 2010) and mangrove roots (August 2009–2012). The eight other common native species had negative relationships with Exaiptasia sp. on mangrove roots and ONS. Suberea sp. 2 showed consistent negative correlations of medium to large effect sizes (≥0.5) with the Exaiptasia sp. on mangrove roots (March and August 2009, August 2010) and on
ONS (March and August 2009). *C. verticillata*, cyanobacteria (on ONS in August 2009–2012 and on mangrove roots in August 2009), *Haliclona* sp. 13 (on ONS in March 2009) and *S. peleia* (on ONS in August 2010 and 2012) were the only four native species with positive relationships with *Exaiptasia* sp. The percent cover of *Exaiptasia* sp. and native diversity were significantly negative correlated with weak effect (-0.5 < r < -0.3) within the mangrove habitat for the first two surveys. Correlations were insignificant between Shannon–Weaver Index values and *Exaiptasia* sp. percent cover within ONS for all four surveys and on the mangrove roots for August 2010 and 2012 (Table 2).

**DISCUSSION**

In six years (2003–2009), the introduced anemone *Exaiptasia* sp. expanded rapidly from a few isolated patches in the side–arm of OTM to fully encompass the lake’s perimeter, dominating substrates such as mangrove roots and rocky slopes. This type of rapid expansion and dominance has become the archetypal ‘invasion’, as in algal invasions of *Caulerpa racemosa* in Cyprus and *Undaria pinnatifida* in New Zealand (Schaffelke & Hewitt 2007; Galil 2007). However, by 2012, the introduced anemone reached its lowest abundance since establishment, which may be part of a boom–bust cycle in which population explosions are followed by a reciprocal decline (Simberloff & Gibbons 2004). Though there have been some significant negative correlations between the abundance of the introduced anemone and some native species, the trajectory for the impact of *Exaiptasia* sp. on the native community is still unfurling through the interplay of a range of biotic and abiotic factors.

**2003: Introduction and establishment**

The location of the first patch of *Exaiptasia* sp., directly in front of the visitors’ entry dock, and the frequent occurrence of other non–native species at this location, strongly suggests that the sea anemone was most likely introduced by a visitor, perhaps as a hitchhiker on a shell or rock, rather than being a natural colonization. Other explanations are all more improbable; for example, the major conduits into OTM through which natural colonization could occur are in other locations in the lake (Figure 2A), cormorants which fly among locations are far less numerous than tourists, and some non–native specimens found by the dock in the lake were too large to be transported by currents or by birds (e.g. the sand dollar found in OTM in 2009). These simple facts constrain the introduction of *Exaiptasia* sp. to between the late–1980s, when tourism to OTM began, and some time shortly preceding the discovery of *Exaiptasia* sp. in 2003.

Colonization processes can take a variety of trajectories (Zenni & Nunez 2013) making it difficult to pinpoint the exact time of introduction. However, several factors—patterns of tourism, observations of other introductions, *Exaiptasia* sp. life history, and symbiont dynamics—may enable us to narrow down the most likely period of introduction. An assessment of these factors to determine the arrival of *Exaiptasia* sp. can also contribute to our understanding of the risk of introductions to OTM.

Tourists or visitors to OTM are potential vectors for introductions into OTM, and the patterns in tourism are important in determining the propagule pressure on OTM. The trend in visitor numbers to OTM is proportional to the number of visitors to Palau.
Tours to OTM began in the late 1980s (Etpison 1997), when visitor numbers to Palau averaged ~10,000 (Yamashita 2008). From 1980 to the early 1990s, the tourist market was dominated by divers—tourists who stayed for a long duration (5–10 days) and are more aware of issues of marine conservation (Yamashita 2008). By 1995, the tourism industry began shifting towards “general tourism.” Tourists who fell under the “general tourism” category doubled in numbers, leading to an increase in total visitor numbers to Palau (~50,000 visitors in 1995; Yamashita 2008). “General tourists” travelled in large groups on packaged tours, stayed for a short duration (3–4 nights), and were less “eco–conscious” (Yamashita 2008). The increase in “general tourists”, and consequently total visitors to Palau, marked the increase in visitors to OTM. Considering that total visitors to Palau averaged ~10,000 in the late 1980s to early 1990s, it can be assumed that less than 10,000 tourists per year visited OTM during that time period. As such, as visitor numbers to Palau increased so did visitors to OTM. In 1994, there were an estimated 30,000 visitors to OTM (Hamner 1994), and by 1997, an estimated ~40,000–50,000 tourists visited OTM (Dawson et al. 2001). A similar trend is seen in more recent visitor records: ~60–70% of 118,754 visitors to Palau visited OTM in 2012 (Koror State Visitor Logbooks for 2012, unpublished).

The increase in the total number of visitors and peaks of general tourists very likely increased propagule pressure as tourists may carry non–native species into OTM. Introductions are even more likely when tourist behavior is taken into account. This includes being less “eco–conscious,” visiting numerous sites before entering OTM, and travelling in large groups that are not carefully monitored. Peaks of general tourists occurred in ca. 1996–1997 and ca. 2002–2003 (Yamashita 2008), giving an estimate of increasing risk in the few preceding years. In addition, observations of other introductions, e.g. sightings of shells and corals, in front of the dock increased in frequency from 1998–2002 (L. Bell, M.N Dawson, pers. comm).

Similarly, the reproductive traits of Exaiptasia, the abundance of which the introduced anemone was first discovered, and the subsequent rate of expansion may be most consistent with introduction occurring shortly before, possibly as little as six months to a year prior to, 2003. Exaiptasia sp. can reproduce asexually through pedal laceration, and in a laboratory experiment, a single Exaiptasia individual can produce 255 individuals through pedal laceration in 77 days (Schlesinger et al. 2010). The rate of expansion of the Exaiptasia sp. ‘front’ (see next section) is evidence of Exaiptasia sp. ability to reproduce at a fast rate. However, the uncertain lag time between introduction and establishment when a newly introduced species adapts to a new environment to become a viable population could expand the timing of introduction from ≤1 year to as much as five years to a decade (Zenni & Nunez 2013; Simberloff & Gibbons 2004), and so include a late–1990s introduction.

The genetic analyses of Symbiodinium, though, suggest the symbiont community is still in the period of establishment, due to apparently considerable lineage sorting over the past decade, during expansion of the Exaiptasia sp. population. If establishment includes a period of selection and adaptation, then it seems we have observed this process in the symbiont population, suggesting we first caught the population really quite early. Exaiptasia sp. is capable of taking up other symbionts, however under a normal environment they will favor and revert to their existing symbiont (Belda–Baillie et al. 2002). Usually, Exaiptasia are associated with Symbiodinium minutum (Type B1) and Symbiodinium sp. "A4" (Thornhill et al. 2013), and they are sensitive to elevated
temperatures (Goulet et al. 2005). Clade D association with \textit{Exaiptasia} sp. is considered atypical, as the commonly reported associations are with clade B and A. Our preliminary results of symbiont population genetic dynamics indicate that \textit{Exaiptasia} sp. reverted from the atypical Clade D association toward the common Clade B association, consistent with an apparent increase in bleaching in \textit{Exaiptasia} sp. in 2012 (S. Patris pers. obs.).

Thus, the timeline for introduction of \textit{Exaiptasia} sp. is suggested, by all four pieces of circumstantial evidence, to be a relatively recent introduction, perhaps best measured in multiples of several months rather than multiples of several years before the discovery in late 2003. Furthermore, these four factors—tourist numbers, reproductive traits and life history, the state at the initial discovery, and the genetics of symbionts—all play important roles in determining risks of introductions and invasions into OTM, and a more in–depth analysis of these factors is necessary to fully understand the risks of future introductions into OTM.

\textbf{2003–2008: Population expansion}

\textit{Exaiptasia} sp., once established, spread around the lake at a fast and increasing rate, overtaking hard substrates such as mangrove roots. \textit{Exaiptasia} sp. took 3 years to fully cover preferred hard substrate in the side–arm, and to spread more than 100m away along the edge from the initial zone of discovery. \textit{Exaiptasia} sp. took only another 3 years to spread a further 500 m and to fully encompass the perimeter of the lake, evidence of an increasing rate of expansion through time as seen in other invasive species (e.g. Ricciardi & Cohen 2007). Subsequently, however, we observed fluctuations in \textit{Exaiptasia} sp. abundances between 2009 and 2012.

The establishment and expansion of \textit{Exaiptasia} sp. in OTM may be attributable to several circumstances. Most notable of these may be that marine lakes, as a newly recognized type of ‘island’, are similarly susceptible to invasions as oceanic islands; they share the same characteristics of low diversity but high endemism (Hamner & Hamner, 1998; e.g. Fautin & Fitt 1991; Dawson & Hamner 2005) and few predators (Hamner 1982; Hamner et al. 1982; cf. e.g., Vitousek et al. 1997; Sax & Gaines 2003). For example, known predators of \textit{Exaiptasia}, such as nudibranchs and puffer fish (Okey et al. 2003), are absent from OTM. These apparently favorable conditions in OTM, coupled with the rapid asexual reproductive potential of \textit{Exaiptasia} sp., and facilitation by its symbiont could have contributed to the establishment and expansion of \textit{Exaiptasia} sp. in OTM. Mutualism, as evinced in the symbiosis of non–native plants and mycorrhizal fungi, can facilitate invasion and expansion by enabling invaders to overcome natural biotic barriers (Richardson et al. 2000; Pringle et al. 2010).

Already, \textit{Exaiptasia} sp. is suspected to be introduced throughout the Pacific (Hawaii, Japan, Mexico; Thornhill 2013). However, rapid expansions and abundances similar to OTM have only been reported in two other areas, in fact two islands: in the Galápagos (Okey et al. 2003) and in Hang Du I, a marine lake in Ha Long Bay, Vietnam (Cerrano et al. 2006). This suggests that island characteristics—such as low diversity, absence of predators—interacting with other factors such as facilitation by a symbiont, may each or all contribute to the successful invasion of \textit{Exaiptasia} sp. However, the three currently invaded island locations may also have other attributes that promoted invasion that we did not measure, such as resource availability and whether conditions
of the new environment match those of the original environment (Davis 2005; Souza et al. 2009).

2009–2012: Occurrence and possible impacts

Exaiptasia sp. abundance was spatially heterogeneous and fluctuated with time, excepting 2012, this introduced anemone was one of four predominant species. Exaiptasia sp. was more abundant on mangrove roots than on ONS, suggesting its impacts should be seen mostly on native organisms also found predominantly on mangrove roots. In fact, native diversity was consistently lower on mangrove roots than in ONS habitat across all surveys, and the March 2009 survey had the lowest Shannon–Weaver Index (mean and median values) when Exaiptasia sp. abundance was highest. However, while significant correlations between Exaiptasia sp. and Shannon–Weaver Index were found only in comparisons on mangrove roots, effect sizes were relatively small (−0.2 ≥ r ≥ −0.4).

The Exaiptasia sp. invasion in OTM is a relatively recent event—compared to several centuries of invasions in terrestrial and marine systems—and its ultimate impact on diversity may not yet be evident if it is still in the early stages of invasion (see Simberloff 2014). Insufficient time may have passed to infer if it will lead to loss of biodiversity or any other negative impact (Forrest & Taylor 2002, Davis 2003, Sax & Gaines 2003), phenomena referred to, respectively, as “extinction debt” (Sax & Gaines 2003) and “invasion debt” (Simberloff 2014). Impact on native species can take decades to detect, as in the case of introduced nitrogen–fixing shrubs and Sargassum alga, often because long–term effects are facilitated through modifying ecosystem functions and habitat alteration, processes that can occur on timescales of several decades (Olabarria et al. 2009; Simberloff 2014). In contrast, some case studies covering centuries of NIS introductions have shown that NIS may not lead to loss of biodiversity. A large number of introductions have been reported in the Red Sea since 1869, but only one extinction of native species has been detected (Mooney & Cleland 2001). Despite centuries of introductions of NIS plants and animals into New Zealand, only 3 native plants have become extinct; note, though, that native bird populations have been decimated by NIS predators (Sax & Gaines 2003). NIS plants in Great Britain, introduced thousands of years ago, have not driven native plants to extinction (Thomas & Palmer 2015). The wide array of literature covering different timescales (decades vs. centuries), NIS species (competitors vs. predators), and regions and habitats (temperate grasslands vs. tropical oceans) depicts the idiosyncrasies and complexity of invasion biology, limiting our ability to predict NIS impact.

In the case of OTM, although only time can tell if Exaiptasia will have major impacts on the persistence of native species, there may be subtler short-term effects. For example, the abundances of Exaiptasia sp. and a subset of constant native species are significantly correlated in OTM. Generally for both types of correlations, significant correlations were found in the first three surveys and on mangrove roots when and where Exaiptasia sp. percent cover was highest. All significant relationships between Exaiptasia sp. and native sponges on mangrove roots were negative, which may indicate competition for space. The only native sponges that had a significant positive relationship with Exaiptasia sp. were on ONS, i.e. S. peleia and Haliclona sp. 13; while this may suggest a positive interaction, it also may simply reflect an indirect relationship tied to shared exploitation of rocky substrate. Likewise, the positive relationship between
Exaiptasia sp. and C. verticillata may result not from direct facilitation but rather from these photosynthetic species’ shared preferences for sunlight. Though Exaiptasia sp. may not be a major driver of diversity at this stage, there is indirect evidence of interactions (mostly negative, but some positive) between the abundances of the non-native and some native species.

The introduced anemone may currently play only a small part in dynamics in OTM because other factors may affect the assemblage on a larger scale. We have focused on Exaiptasia sp. as a competitor for space in OTM and, though there is evidence of interactions with native species, the impact on diversity is not easily detected. Research on newly introduced species that compete for resources, such as food and space, have not indicated loss of biodiversity or negative impact on native species’ abundance (Olabarria et al. 2009). Introduced predators and pathogens are more likely to cause extinctions than are introduced competitors (Gurevitch & Padilla 2004, Davis 2003). Introduced marine predators [e.g. Mnemiopsis leidyi, comb jellies in the Black sea, (Shiganova 1998) and Carcinus maenas, a green crab in Bodega Bay Harbor, California (Grosholz et al. 2000)] can severely limit resident species abundance and distribution, and at times lead to fishery collapse (Bax et al. 2003). In addition, loss of biodiversity is often attributed to invasive species being the primary antagonist; however, loss of biodiversity is also compounded by other factors such as habitat alteration and human predation (Sax & Gaines 2003, Davis 2003).

**Conclusion and Implications: Conservation and management of marine lakes**

We have established that Jellyfish Lake (OTM), an isolated marine lake with low diversity, high endemism, and few predators, is susceptible to invasions through the case study of the introduced Exaiptasia sp. sea anemone. The rapid expansion, though aesthetically alarming, had very little measurable impact on native diversity at the early stages of this relatively recent invasion. However, future introductions and interactions of non-native species, habitat alteration and degradation, and natural environmental changes are just a few of the many factors that could change the current native–exotic relationships in OTM (see Sax and Gaines 2003; Davis 2003; Fridley et al. 2007).

Though our study found little evidence of strong impact of Exaiptasia sp. on native biodiversity, other case studies of NIS have described ecological and socio-economic impacts of non-indigenous species (NIS; see Vitousek et al. 1997; Reaser et al. 2007; Molnar et al. 2008). This provides a quandary for management, to act or not act on the prevention of NIS introductions. From a socio-economic aspect, the uncertainty of a strong effect may be outweighed by the potential impact; the stakes may be too high to not act to minimize the likelihood of NIS. The economy of Palau is largely reliant on tourism (Osman 2004, Yamashita 2008), and with the recent increase of tourists to Palau, the risk of NIS introductions is increasing. In 2012, 118,754 tourists visited Palau, and ~60-70% visited OTM (Palau Visitors Authority 2012–2013 Visitor Arrival report & Koror State Government visitor 2012 logbooks: unpublished data). The large percentage of visitors to OTM is indicative of not only the recent sheer volume of visitors to this lake, but also its market value in the tourist industry as a popular site for swimming with jellyfish. The increase of tourists has increased propagule pressure, and because predicting introductions of NIS and their impact is difficult (Simberloff 2014), Koror State Government may wish to evaluate whether management efforts should focus on NIS.
introductions into this key revenue–generating tourist site. Prevention of NIS is easier to monitor and enforce, while eradication of NIS is time–consuming and expensive (Vitousek et al. 1997; Reaser et al. 2007). For now, the appearance but not the diversity of the benthos has changed measurably in OTM. However, the unpredictability of future introductions and the sustained human pressure on this natural resource, may very well lead to unexpected, and probably unfavorable, outcomes. At least, the case study of the *Exaiptasia* sp. introduction into OTM should be applied as a precautionary tale to other marine lakes of Palau and other countries, warning of how tourists can affect these isolated marine habitats through NIS introductions.
Figure 1. Data from the eight line transects in the side-arm of OTM surveyed from 2003 to 2006. Transect #1 (T1) is immediately in front of the visitor’s dock, and subsequent transects (T2–T8) are spaced at ~6 m intervals. The x-axis shows distance along transects in an approximately west-east orientation. The y-axis shows depth (meters). Sampling intervals of 2 m are indicated by light grey dots, and *Exaiptasia* sp. presence is indicated by a black circle, the area of which is proportional to *Exaiptasia* sp. abundance; the scale at the top right corner represents the proportion of *Exaiptasia* sp. abundance, i.e. the number of squares in each quadrat with at least one *Exaiptasia* sp. individual.
Figure 2. Distribution of *Exaiptasia* sp. in OTM from 2003–2012. Plots are imposed on a bathymetric map of OTM, with indication of depths at 10, 20, and 30 m. (A) In 2003, *Exaiptasia* sp. was found only in the side–arm near the dock (marked in NW corner) where tourists enter the lake. Colored lines represent the subsequent expansion of *Exaiptasia* sp., marking the furthest extent of the continuous line from 2004–2007. Tunnels in OTM are marked with ‘Xmaj’ for large water flow and ‘Xmin’ for minimal water flow. (B) Bubble plot indicates the scoring of patches from 2009–2012, with different colors representing different survey dates. Bubble sizes are proportional to the scoring of sites based on 20% cover intervals of *Exaiptasia* sp., i.e. 0 is for no *Exaiptasia* sp., 1 is for 20% cover, 2 is for 40% cover, and so on.
Figure 3. Photo–quadrat survey results of (A) Exaiptasia sp. percent cover and (B) Shannon–Weaver Index for each habitat (white=ONS, grey=mangrove roots) per survey (March 2009, August 2009, August 2010, August 2012). Other near surface substrate (ONS) habitat is the category of all other substrates that are not mangrove roots; this includes rocks, mud and fallen trees. Whiskers represent the range of values, circles found outside the whiskers are outliers, thick black lines in boxplots are median values for each habitat group. Mean (x) and median (Md) values per survey are reported in the top of each survey box.
Table 1. Pearson’s coefficient (r) values for correlations between percent cover of native species and Shannon–Weaver Index calculated for native taxa alone (response variables) versus *Exaiptasia* sp. (predictor variable) within habitats (mangrove roots or other near surface substrate [ONS]) per survey (March 2009, August 2009, August 2010, August 2012). Data collected via photo–quadrat surveys and represent subset of "constant" species. Significant correlations (p<0.05) highlighted grey; no Bonferroni corrections were made to p-values. Tests achieve 0.5 < power < 0.8 when magnitude 0.4 < r < 0.6. A mark of “—” indicates that species were not found in surveys.

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Table 2. Spearman rho values for correlations between percent cover of native species and Shannon–Weaver Index calculated for native taxa alone (response variables) versus *Exaiptasia* sp. (predictor variable) within habitats (other near surface substrate or mangrove roots) per survey (March and August 2009, August 2010 and 2012). Data collected via photo–quadrat surveys. Significant correlations (p≤0.05) are highlighted grey; no Bonferroni corrections made to p-values. A mark of “—” indicates that species were not found in surveys.

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<th>Phylum</th>
<th>Species</th>
<th>ONS Mar-09</th>
<th>Aug-09</th>
<th>Aug-10</th>
<th>Aug-12</th>
<th>Mangrove Roots Mar-09</th>
<th>Aug-09</th>
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<td>-0.470</td>
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REFERENCES


Shiganova TA (1998) Invasion of the Black Sea by the ctenophore Mnemiopsis leidyi and recent changes in pelagic community structure. Fish Oceanogr 7:305–310


Thomsen MS, Wernberg T, Olden JD, Byers JE, Bruno JF, Silliman BR, Schiel DR (2014b) Forty years of experiments on aquatic invasive species: are study biases limiting our understanding of impacts? Neobiota 22:1–22


