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RESEARCH ARTICLE

Expansion of an introduced sea anemone population, and its associations with native species in a tropical marine lake (Jellyfish Lake, Palau)

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Abstract. Understanding the full range of consequences of species introductions into island and marine habitats requires quantitative studies of systems that are currently under-represented in the scientific literature. We document the introduction, proliferation and establishment of a non-native sea anemone species in an isolated tropical marine lake, a marine ‘island’. From 2003–2012, we gathered samples to identify the introduced species and used transect and photo-quadrat surveys to describe its abundance, distribution, and any associations with native species or habitats. The non-native sea anemone was first found at the tourist entry into the lake in 2003 and identified as *Exaiptasia pallida* (Agassiz 1864), a species with zooxanthellae endosymbionts. Temporal patterns of tourism, the spatial extent of the sea anemone in 2003, and genetic analyses of the symbiont were consistent with the early stages of introduction. Subsequent expansion of *E. pallida* throughout the lake occurred within six years. The native species assemblages that were invaded by *E. pallida* were heterogeneous among surveys and habitats. Overall, there were few correlations that were significant between percent cover of *E. pallida* and native species; most significant associations were negative; the majority were on mangrove roots. There was one positive association between *E. pallida* and a native sponge. No significant relationship was found between the abundance of *E. pallida* and native species diversity. The rapid expansion of *E. pallida* but dearth of strong ecosystem effects presents a case study of invasive species in a tropical marine habitat where consequences are not directly proportional to invasive abundance. Whether this outcome is stable and representative of other species introduced into marine lakes, or elsewhere in marine systems, remains to be seen.

Keywords. Anemone, biodiversity, invasive species, island, marine lakes, non-indigenous species (NIS), Palau

Introduction

Non-indigenous species (NIS) and their negative or positive associations with native species, ecosystem functions, and society have been much debated in descriptions of species invasions (Bax et al. 2003, Davis 2003, Sax and Gaines 2003, Galil 2007, Molnar et al. 2008). For a number of historical reasons—including heterogeneity in economics and the establishment of research facilities and personnel—these debates on NIS are biased geographically towards temperate regions. Few studies address tropical or polar regions, and even fewer focus on marine habitats

(Fridley et al. 2007, Molnar et al. 2008, Thomsen et al. 2014). Of the available literature describing marine introductions, sessile organisms—such as molluscs, algae, and ascidians—are the most widely reported, while non-native marine fishes and cnidarians are under-represented (Molnar et al. 2008, Thomsen et al. 2014). Situations in which invasions have been common include semi-enclosed bays, estuaries, and seas (e.g., Hewitt et al. 2004), but because such coastal locations have experienced heavy ship traffic, human development, and waves of introduced species over the last century (Galil 2007), the introductions themselves can be difficult to study. The ‘open’

nature of such ecosystems (i.e., few clearly defined boundaries and high dispersal potential of many marine organisms), the prevalence of cryptogenic species, and difficulties of taxonomy make it challenging to document, identify, and therefore predict the expansion of introduced marine species (Carlton 1996, Holland 2000, Dawson et al. 2005).

The challenges of studying marine introductions are greatly reduced in marine lakes—isolated bodies of seawater entirely surrounded by land (Dawson and Hamner 2005). Marine lakes offer an opportunity to study ecological and evolutionary processes influencing marine NIS in a setting that is analogous to terrestrial islands (Dawson 2015, Hachich et al. 2015). Marine lakes have an advantage over bays, estuaries, and seas because marine lakes are largely new to exploration and exploitation. Thus, if, or inevitably when, introductions happen, 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.

In 2003, we discovered a non-native sea anemone introduced in Jellyfish Lake (Ongeim'l Tketau), a tropical marine lake in Palau (Figs. 1, 2). This was the first documented introduction of any organism into a marine lake, offering an opportunity to lay the foundations to explore several questions. For example, in marine islands, how does an introduction progress? Do introductions monopolize space with an associated decrease in endemic species diversity, as in introductions

of algae (Schaffelke and Hewitt 2007), or is there little evidence of exclusion (Sax and Gaines 2003; Thomas and Palmer 2015) indicating 'accommodation' and increase in local biodiversity (Briggs 2010)? Particularly, do the earliest stages of invasion foreshadow any of these divergent outcomes? Thus, the primary goals of this study were to (1) describe the discovery of the initial introduction, and (2) measure the abundance and distribution of the introduced species of sea anemone, as well as native lake species, in Jellyfish Lake through time.

Methods

Study site

Jellyfish Lake, or Ongeim'l Tketau (OTM), on the island of Mecherchar in Palau (Fig. 1), is a closed and isolated lake completely surrounded by jungle-covered karst, well-known for its unique perennial population of millions of golden jellyfish (Colin 2009). 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. 2006) and a jellyfish population crash following the extreme 1997–1998 El Niño–La Niña (Dawson et al. 2001). Though not as species rich as reef communities, OTM has a high proportion of endemic populations and species (Fautin and Fitt 1991, Hamner and Hamner 1998, Dawson and Hamner 2005). Because of its unique endemic population of *Mastigias* jellyfish, OTM has

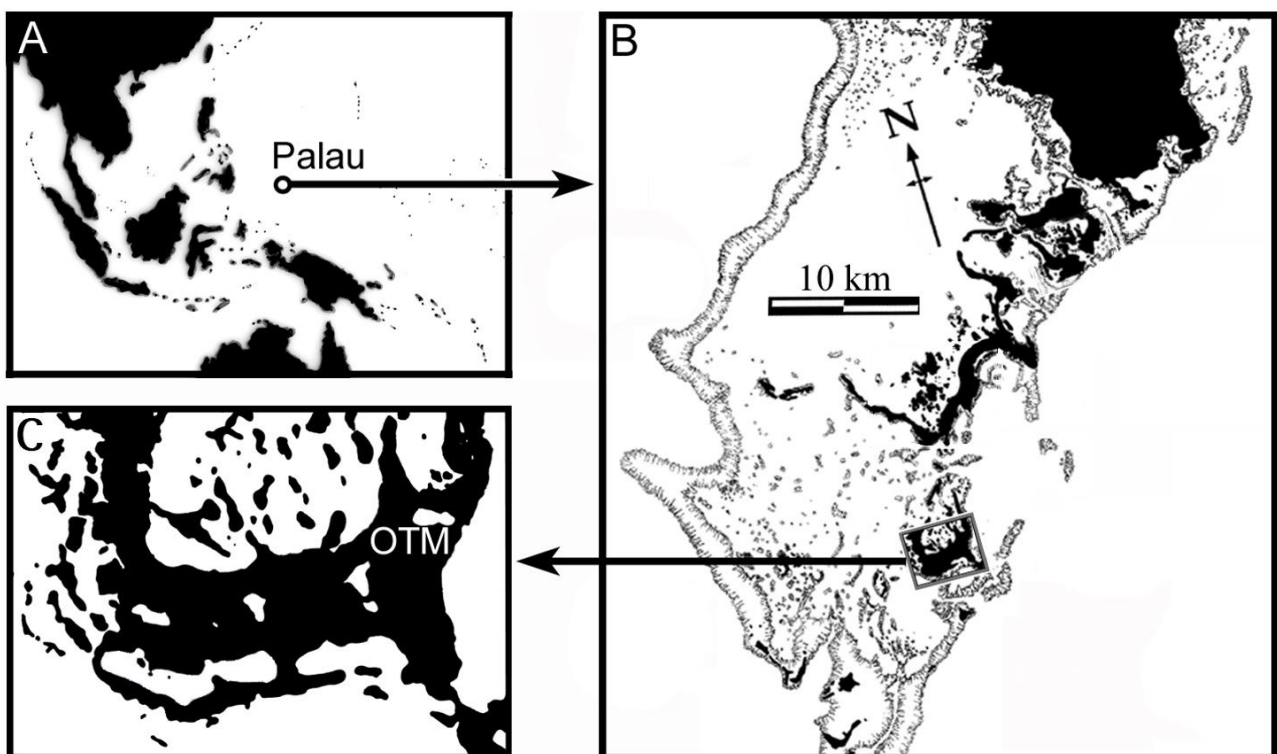


Figure 1. Map of the study regions and location. (A) The location of Palau within the Western Pacific. (B) The island on which Ongeim'l Tketau, Jellyfish Lake, is located within Palau. (C) The location of Jellyfish Lake, the eastern-most lake on the island of Mecherchar, Palau.

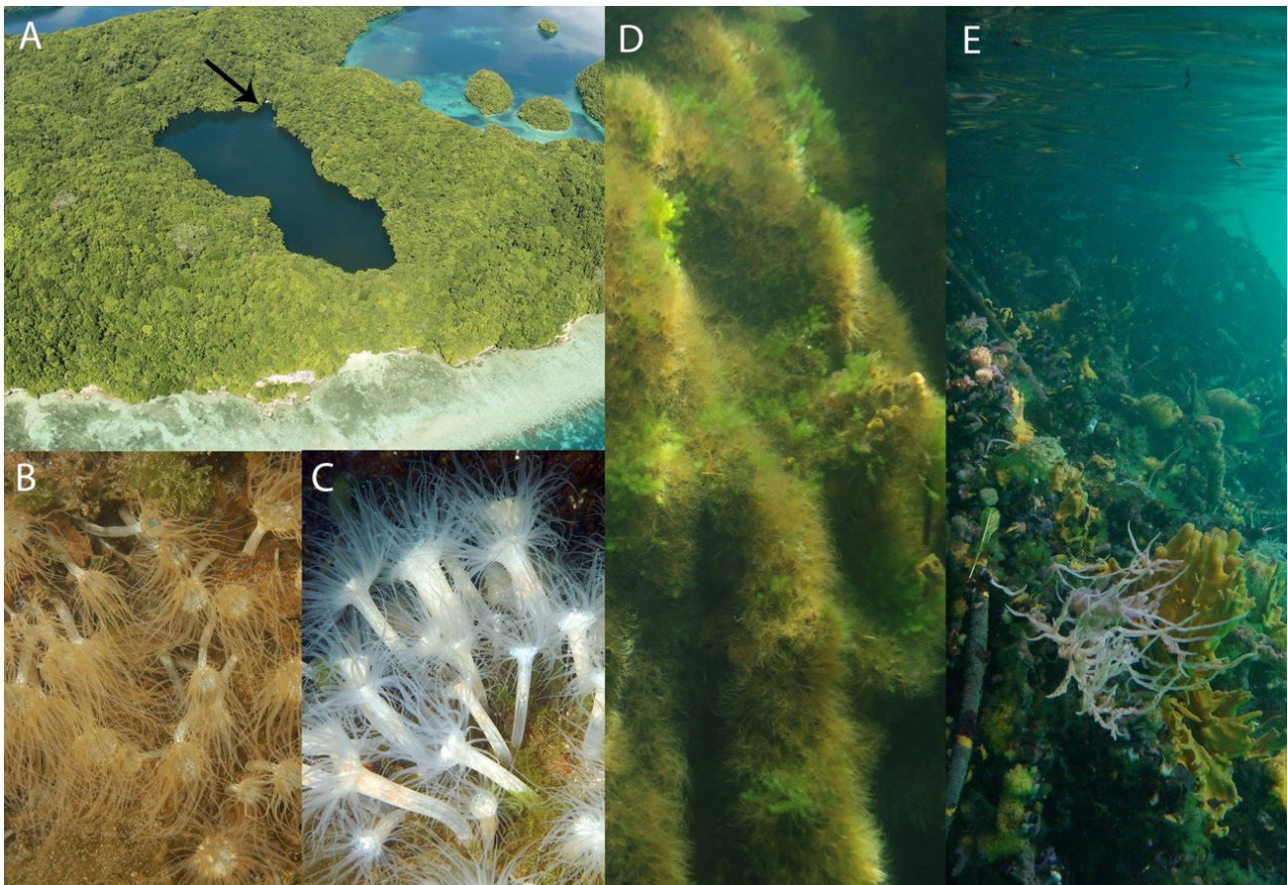


Figure 2. (A) An aerial photograph of Jellyfish Lake, showing its physical isolation from the ocean (photo courtesy of Mandy Etpison). Black arrow points to side-arm of lake, where the entry dock is located and where the first introduction of the sea anemone, *Exaiptasia pallida*, was discovered and documented, see Figure 3. (B) The introduced *Exaiptasia pallida* contrasted with (C) the endemic sea anemone, *Entacmaea medusivora*, in Jellyfish Lake. (D-E) Photographs of mangrove roots with and without *E. pallida* in Jellyfish Lake.

become an important economic resource—garnering millions of dollars annually from the heavily marketed tourist industry, one of Palau’s main income resources. However, the increase of visitor traffic has opened OTM to an elevated possibility of introductions of non-native species.

Discovery of introduction, November 2003, and identification

On 14 November 2003, a patch of sea anemones—morphologically distinct from the native sea anemone *Entacmaea medusivora* (Figs. 2B, 2C)—was discovered in a side-arm of the lake in front of the dock from which tourists access the lake. We collected specimens, sent them to specialist taxonomists for identification, and requisitioned DNA sequencing of the host and zooxanthellae (see Supplementary Documentation I. Sequencing of *Exaiptasia pallida* and its zooxanthellae).

Quantitative Surveys, 2003 to 2007

From 2003–2007, we documented the spread of the novel sea anemone from the point of discovery in the side-arm of the lake and subsequent expansion into the

rest of the east basin. Beginning in 2003, we conducted quantitative surveys of the novel sea anemone in the side-arm of the lake by documenting the occurrence and density of introduced sea anemones on eight parallel transects. Transect #1 (T1) was immediately in front of the visitor’s dock, and subsequent transects (T2–T8) were spaced at ~6 m intervals. Whenever we encountered a sea anemone along the transect, we placed a 0.5 x 0.5 m quadrat against the transect line and enumerated the number of 10 x 10 cm squares within the quadrat that had at least one sea anemone. If the patch of sea anemones 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. The original method was modified slightly in 2004–2006 due to a substantial increase in coverage of the sea anemone; from 2004–2006, the quadrat was placed every two meters from 0 m to the end of each transect.

In addition, we visually inspected areas of the lake adjacent to the side-arm and noted patches of introduced sea anemone found outside the side-arm, in the main basins of the lake. We expanded the survey

to encompass the whole lake in 2006 and 2007 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 distribution of the introduced sea anemone). The continuous distribution was defined as being a relatively uninterrupted density of introduced sea anemones with gaps less than 10 m between patches. When the nearest neighboring patch of sea anemones was separated from the current patch by more than 10 m distance, that discontinuity established the position of the 'front' separating the continuous distribution from satellite colonies.

Quantitative Surveys and Association Analyses, 2009 to 2012

Due to the rapidly increasing distribution of the sea anemone, we transitioned to conducting two new types of surveys in March 2009: (1) transect surveys and (2) photo-quadrat surveys. All surveys were conducted through snorkeling and circumscribed the entire lake. We repeated these surveys in August 2009, August 2010, and August 2012.

(1) *Transect surveys: density, distribution and extent of the non-native sea anemone* — Transect surveys documented density, distribution and extent of the non-native sea anemone along the perimeter of OTM. At each 10 m interval on a transect tape, we searched for non-native sea anemones in a ~5 m² 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 10 m from the perimeter) were re-mapped onto the shoreline of the lake between the immediately preceding and following data points.

(2) *Photo-quadrat surveys: abundance and associations of the non-native sea anemone* — Photo-quadrat surveys were conducted to determine abundance and associations of the non-native sea anemone, substrates, and endemic species in OTM. 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 for a total of 4 surveys (see dates, above), each survey having new random positions. During each survey, a 0.25 x 0.25 m quadrat was laid out, at 1–3 m depth, and photographed at 60 random sites along the lake's perimeter.

Percent cover of species was calculated using the random point count methodology through the Coral Point Count with Excel extensions (CPCe; Kohler and 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.

We estimated the species composition of the benthic assemblage in two ways. First, we used the relative frequencies of quadrats in which a species was present for each species in each survey. Second, we calculated the mean percent cover per survey. We determined that the two estimates were statistically correlated ($R = 0.74$). Therefore, we categorized species as constant (present in all four surveys) or not (missing from at least one survey) and as predominant (occurs in $\geq 50\%$ of quadrats), common (occurs in 20–50% of quadrats), or rare (occurs in $\leq 20\%$ of quadrats) for further analyses.

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 and Warwick 2001). To determine the relative habitat composition of the lake, habitat under each quadrat was identified as mangrove roots, rock, fallen tree, mud or algae. The occurrence of the invasive sea anemone (i.e., the number of quadrats in which the invasive sea anemone was found in each survey) was then plotted relative to habitat availability to evaluate any habitat preferences.

Because some habitats were rare or not observed in all surveys and mangrove roots made up 55% of total sites, habitat was categorized into mangrove roots and other near surface substrate (ONS) for community analysis; ONS encompasses all other substrates that are not mangrove roots. As sites were separated by habitat, we ran permutational multivariate analysis of variance (PERMANOVA; Anderson 2001, Clarke and Gorley 2015) to test differences among surveys within habitats using 999 permutations. In addition, we tested for homogeneity of multivariate dispersions using permutation in PERMDISP (Anderson 2006). SIMPER (Clarke 1993) was used to test which species contributed the most to the similarity matrix within groups and the dissimilarity between groups.

Native species diversity was determined with the Shannon diversity index (H), which calculates species diversity based on their abundance and evenness. To understand factors determining native diversity and non-native abundance, including relationships between the two, we first ran a two-way ANOVA for (i) Shannon–Wiener Index calculated for all native species in each quadrat and (ii) the percent cover of introduced sea anemones using factors of survey dates and habitat and their interaction. ANOVA is robust to deviations from normality if the assumption of homogeneous variance is not violated, which we tested using Cochran C–Test revealing no significant difference in homogeneity (Cochran–C Test = 0.17, $p = 0.75$). Non-native sea anemone percent cover was $\log(X+1)$ transformed to remove heteroscedasticity. 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–Wiener Index.

Finally, we used Spearman rank correlation to describe relationships between the non-native sea anemone and (a) overall native diversity and (b) the ‘constant’ native species for each survey within each habitat. Significant correlations ($p < 0.05$) were further tested using the sequential Bonferroni correction ($p < 0.1$).

Results

Discovery of introduction, November 2003, and identification

The first discovery of the non-native sea anemone was of a $\sim 3 \text{ m}^2$ patch at $\sim 3 \text{ m}$ 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 DNA sequencing (GenBank accession numbers MK334667–MK334681; see Supplementary Documentation I. Sequencing of *Exaiptasia pallida* and its zooxanthellae). Five anemone specimens were identified as *Exaiptasia pallida* (Agassiz in Verrill, 1864), formerly *Aiptasia pulchella* and *Aiptasia pallida* (Grajales and Rodriguez 2014). Samples of zooxanthellae collected at different times between the years of 2003–2015 were identified as *Durusdinium trenchii*

(formerly *Symbiodinium trenchii*), *Breviolum minutum* (formerly *Symbiodinium minutum*), or *Cladiocopium* sp. (LaJeunesse et al. 2018; see Supplementary Documentation I. Sequencing of *Exaiptasia pallida* and its zooxanthellae for more details).

Quantitative Surveys, 2003 to 2007

Over a period of three years, *E. pallida* spread rapidly, covering mangrove and rock substrate in the side-arm where the visitors’ dock is located (Fig. 3). 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 *E. pallida* 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 $\geq 80\%$ cover of *E. pallida*. Mangrove roots and other hard substrates were covered densely with *E. pallida* (Figs. 2D, 3).

Exaiptasia pallida patches, nearly contiguous with the continuous distribution, were first observed outside the initial survey zone in 2004 along the northern perimeter of the western basin of the lake (Fig. 4A). A year later in 2005, patches of *E. pallida* were found along the western and northern rim, in areas still adjacent to the side-arm. By 2006, the *E. pallida* population formed a continuous line more than 100 m outside the initial invasion area along both the northern and western perimeter (Fig. 4A).

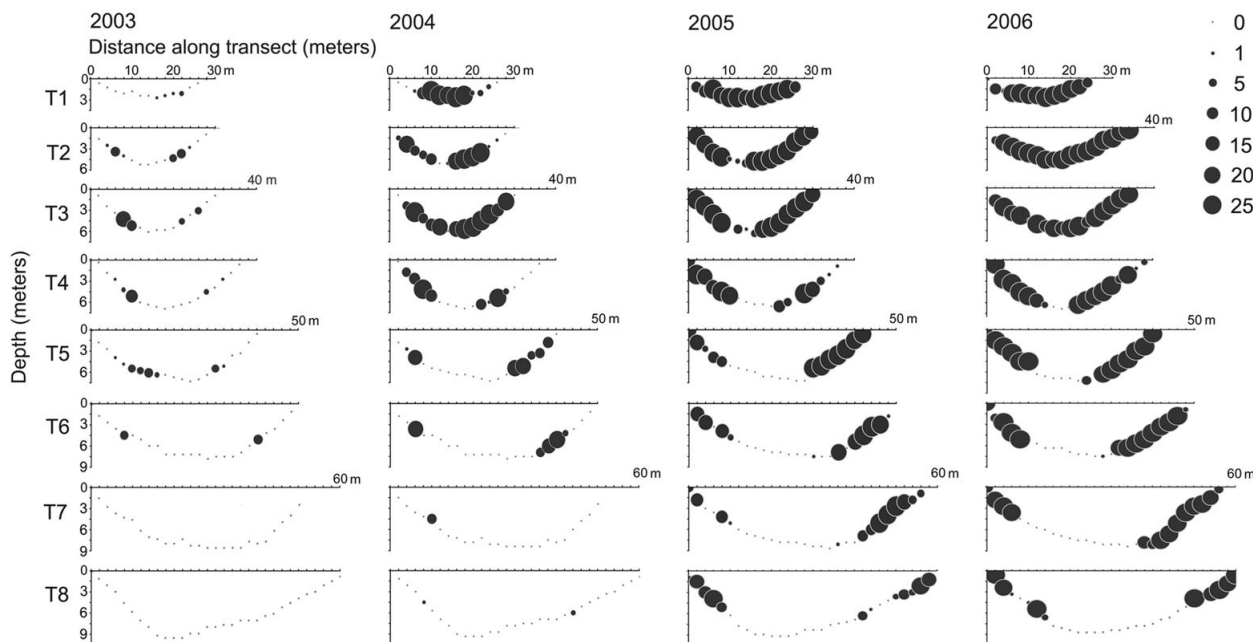


Figure 3. The early stages of establishment and expansion of the introduced population of *Exaiptasia pallida*, documented along eight line transects that transversed the side-arm of Jellyfish Lake (shown in the NW corner of the lake in Figures 2 and 4), from 2003 to 2006. Transect #1 (T1) is immediately in front of the visitor’s dock, and subsequent transects (T2–T8) are spaced at $\sim 6 \text{ m}$ intervals; see Figure 4 for location of Transect 1, 4 and 8. The x-axis shows distance along transects in an approximately west-east orientation. Sampling intervals of 2 m are indicated by light grey dots, and *E. pallida* presence is indicated by a black circle, the area of which is proportional to *E. pallida* abundance. The scale at the top right corner represents the proportion of *E. pallida* abundance, i.e., the number of squares in each $0.5 \times 0.5 \text{ m}$ quadrat with at least one *E. pallida* individual.

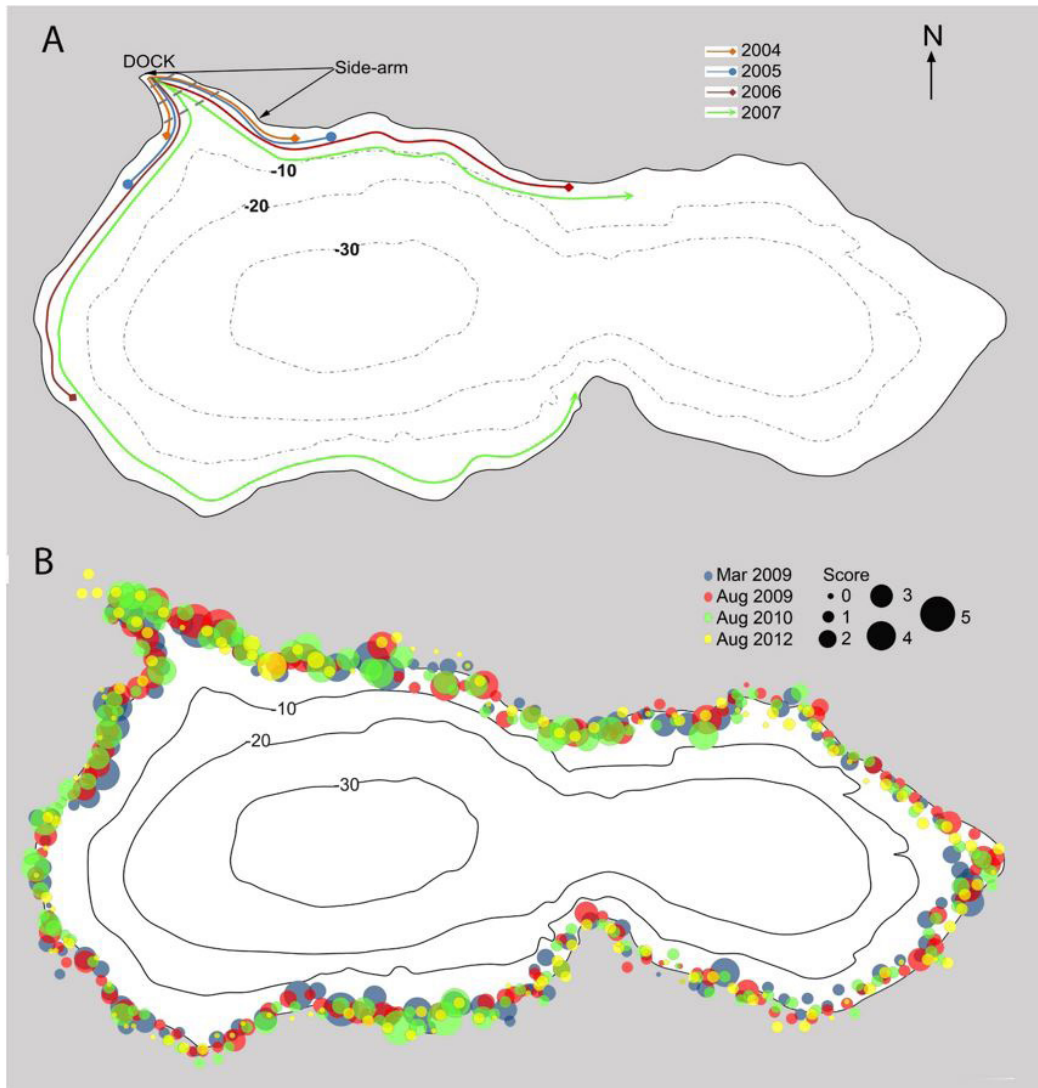


Figure 4. Continued expansion of *Exaiptasia pallida* in Jellyfish Lake from 2003–2012. (A) Colored lines represent the expansion of *E. pallida*, from the original 2003 location in the side–arm near the tourist dock (marked in NW corner) to the farthest extent in each of the subsequent years, 2004–2007. Dashed grey lines near dock located within side–arm represent location of Transect 1, 4, and 8; subsequent transects (T2–T7, not pictured) are spaced at 6m intervals between T1 and T8. (B) Bubble plots indicating the distribution and abundance of *E. pallida* 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 *E. pallida* (i.e., 0 is for no *E. pallida*, 1 is for 20% cover, 2 is for 40% cover, and so on). Line and bubble plots are superimposed on a bathymetric map of the lake with contour lines at depths of 10 m, 20 m, and 30 m.

A few discontinuous ‘satellite’ patches extended past the continuous line in the west basin; only one small satellite patch was found in the east basin. In 2007, *E. pallida* 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 and Association Analyses, 2009 to 2012

(1) *Transect survey: density, distribution and extent of the non-native sea anemone* — In March 2009, *E. pallida* was present throughout the east and west basins of the lake, covering mangrove roots and

hard substrate along the sides of the lake (Fig. 4B). Areas lacking *E. pallida* occurred at a frequency of 1–3% for the first three surveys but jumped to 40% in 2012. For each survey from 2009–2012, over half of the sites had 20% *E. pallida* cover. The number of sites with 40%–60% cover was variable among surveys but showed a decreasing trend from March 2009 to August 2012, which is indicated by decreasing circle (=score) size in Fig. 4B. Sites with scores of 80% and 100% were the least common and further decreased to a frequency of only 1% in August 2012.

(2) *Photo-quadrat survey: abundance and associations of the non–native sea anemone* — Results indicate

that the invasive sea anemone preferentially occurs on hard substrates such as mangrove roots and fallen trees (Fig. 5). However, there was a decline in *E. pallida* percent cover between 2009 and 2012 overall, from a percent cover average of 15% to 2%.

PERMANOVA analysis indicates that the percent-cover of species in OTM changed through time, and that a different assemblage of species was found on mangrove roots and other near-surface substrate (ONS) habitats (Table 1). Moreover, mangrove habitat assemblages showed significant differences among surveys ($F = 2.64$, $p = 0.005$; $P = 0.01$). ONS habitat also showed significant differences among surveys ($F = 5.70$, $p = 0.001$); however, PERMDISP values for ONS habitat were not significant ($P = 0.17$), Supplementary Documentation Table S5). The significant PERMANOVA and PERMDISP values indicated the difference between assemblages according to factors of habitat and survey dates is influenced by the differences in variance within the groups. ONS species' percent cover were similar among the survey dates whereas species' percent cover on mangrove roots were variable.

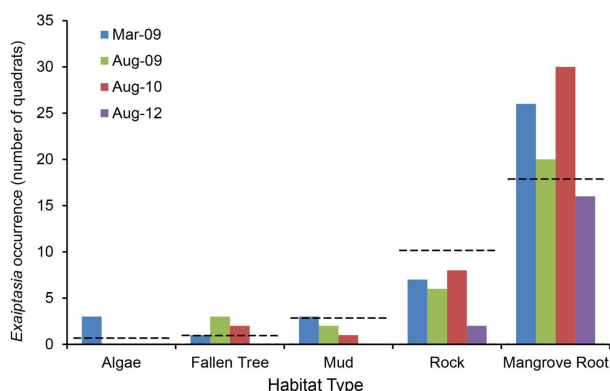


Figure 5. Number of quadrats containing *Exaiptasia pallida*, by substrate and by survey date in Jellyfish Lake (Ongeim’l Tketau, Mecherchar). Dashed black line shows expectation of quadrats containing *E. pallida* if the invasive anemone is randomly distributed in proportion to the availability of different habitat types. The figure shows lower than expected occurrence on rock and mud habitats and higher than expected occurrence on mangrove roots and fallen trees.

SIMPER results indicated that, overall, *Caulerpa fastigiata*, *C. verticillata*, *Brachidontes* sp. 2, and *E. pallida* were the top four species driving similarity averages within surveys and dissimilarity averages among surveys (Supplementary Documentation Table S6). Within mangrove habitats, pairwise comparison between all surveys had over 65% dissimilarity, with the top four species driving dissimilarity averages (Supplementary Documentation Tables S7, S8). In 2012, however, *E. pallida* 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 (Supplementary Documentation Table S6).

Of the 59 known benthic species in Jellyfish Lake, 45 species were present and identified in photo-quadrats, of which only 21 species were ‘constant’, that is, present in all four surveys (see Supplementary Documentation Table S9 for more details). Of the 21 constant species, four were ‘predominant’ (i.e., occurred in ≥50% of total quadrats): the influential two macroalgae, mussel, and the non-native sea anemone (*E. pallida*). Eleven species were ‘common’ (i.e., occurred in 20–50% of quadrats): an ascidian, a gastropod, seven sponges, and cyanobacteria. The remaining six — the native sea anemone and five sponges — plus all other species were identified as ‘rare’ (i.e. in ≤20% of quadrats).

ANOVA revealed a significant difference in Shannon-Wiener Index among survey dates ($df = 3$, $F = 4.95$, $p < 0.01$) and between habitats ($df = 1$, $F = 5.42$, $p < 0.01$). Interaction between survey dates and habitat was not significant ($F = 0.40$, $p = 0.27$). *E. pallida* percent cover was different across survey dates ($df = 3$, $F = 12.61$, $p < 0.001$) and habitats ($df = 1$, $F = 42.78$, $p < 0.001$). The overall mean percent cover of *E. pallida* per survey was highest (15%) in March 2009 and lowest in August 2012 (3%). Higher cover of *E. pallida* occurred on mangrove roots than in other habitats, with the highest mean percent cover on mangrove roots found in March 2009 (23% mean cover) and the lowest mean percent cover on mangrove roots in August 2012 (4% mean cover). For ONS, *E. pallida* mean percent cover decreased in rank order from fallen trees, to algae, mud, and rock habitats. However, *E. pallida* was disproportionately abundant on fallen trees relative to the rarity of the habitat type; *E. pallida*

Table 1. PERMANOVA results for all surveys of *Exaiptasia pallida* and native species abundance in Jellyfish Lake, with date, habitat (mangrove roots and ONS) and their interaction as fixed factors. Differences between dates indicate that percent cover of species changed through time, and differences between habitats indicate that different assemblages are found on the different habitats.

Source	Type	Levels	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Date	Fixed	4	3	35163	11721	5.8529	0.001	999
Habitat	Fixed	2	1	21795	21795	10.883	0.001	998
Date x Habitat	Interaction		3	13081	4360.3	2.1773	0.007	997
Residuals			232	4.65E+05	2002.6			

Table 2. Spearman rho values for correlations between *Exaiptasia pallida* (predictor variable) and percent cover of a subset of native species with at least one significant association or Shannon–Wiener Index of native community (response variables) in Jellyfish Lake, calculated for two habitat categories: other near surface substrate (ONS) and mangrove roots. Correlations are given per survey (March and August 2009, August 2010, and August 2012) to permit visualization of temporal consistency or change in relationships. Predominant (*), common (**), and rare (***) species are marked with corresponding number of asterisks. Significant correlations with sequential Bonferroni correction ($p < 0.1$) are highlighted in grey. A mark of “—” indicates that species were not found on that particular habitat during the survey.

Phylum	Species	ONS				Mangrove Roots			
		Mar-09	Aug-09	Aug-10	Aug-12	Mar-09	Aug-09	Aug-10	Aug-12
Chlorophyta	<i>Caulerpa fastigiata</i> *	-0.156	0.086	-0.306	0.251	-0.429	-0.338	-0.510	0.251
Mollusca	<i>Brachidontes</i> sp. 2*	-0.120	-0.215	-0.193	0.041	-0.207	-0.586	-0.164	-0.338
Porifera	<i>Dendrilla lendenfeldi</i> **	-0.253	-0.318	-0.560	-0.146	-0.363	-0.504	-0.289	-0.204
	Encrusting sponge**	-0.416	-0.306	-0.065	0.084	-0.547	-0.284	-0.341	-0.294
	<i>Spheciospongia peleia</i> ***	-0.235	-0.235	0.114	0.662	--	--	-0.226	--
	<i>Suberea</i> sp. 2**	-0.525	-0.475	-0.287	-0.248	-0.517	-0.501	-0.646	-0.295
	Shannon-Wiener Index	-0.274	-0.261	-0.222	0.083	-0.370	-0.470	-0.182	-0.141

was more common than expected on mangroves given this substrate’s prevalence (Fig. 5).

Spearman rank correlation, with sequential Bonferroni correction, identified eight significant correlations between *E. pallida* and the predominant and common native species. Of the eight significant correlations, two were found on ONS habitat in March 2009 and August 2012, and the other six were found on mangrove roots in March 2009, August 2009, and August 2010 (Table 2). Only one native sponge had a positive association with *E. pallida* on ONS habitat, while the other 7 relationships were negative. Correlations were insignificant between Shannon–Wiener Index values and *E. pallida* percent cover for all four surveys and habitats (Table 2).

Discussion

In six years (2003–2009), the introduced sea anemone *E. pallida* 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 and Hewitt 2007, Galil 2007). However, by 2012, the introduced sea anemone reached its lowest abundance since establishment, which may be part of boom-bust or ‘surges and setbacks’ cycles in which population explosions are followed by decline (Simberloff and Gibbons 2004, Canning-Clode and Carlton 2017). Though some significant negative correlations between the abundance of the introduced sea anemone and certain native species were identified, the trajectory for the impact of *E. pallida* on the native community is still unfurling through the interplay of a range of biotic and abiotic factors. Below we review the rise and fall of *E. pallida* throughout the main stages of its invasion process, following Blackburn et al. 2011.

2003: Transport, introduction and establishment

The location of the first patch of *E. pallida*, 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 into the lake from the surrounding ocean by a visitor, probably as a hitchhiker on a shell or rock carried in by a visitor, or perhaps on snorkel gear or wetsuits, rather than being a natural colonization. Other explanations are all more improbable; for example, the major conduits into OTM, through which natural colonization could hypothetically occur, are in other locations in the lake; 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). Tourism to the lake began in the late 1980s, with peaks of general tourists occurring in ca. 1996–1997 and ca. 2002–2003 (Yamashita 2008). 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.J. Bell, M.N Dawson, pers. obs.). Similarly, the reproductive traits of *Exaiptasia*, the abundance at which the introduced sea anemone was first discovered, and the subsequent rate of expansion seems most consistent with introduction occurring shortly before, possibly as little as six months to a year prior to 2003. In addition, the genetic analysis of zooxanthellae shows considerable lineage sorting over the past decade during expansion of the *E. pallida* population (see Supplementary Documentation I. Sequencing of *Exaiptasia pallida* and its zooxanthellae). This disequilibrium suggests the symbiont community was still in the period of establishment, with possibly multiple introductions of anemones with slightly different endosymbionts.

Thus, the timeline for *E. pallida* is suggested, by all four pieces of circumstantial evidence, to be a relatively recent introduction, perhaps best measured

in multiples of months rather than multiples of years before the discovery in late 2003. These four factors—tourist numbers, reproductive traits and life history, the status at the initial discovery, and the genetics of symbionts—all play important roles in determining risks of future introductions and invasions into OTM. Consequently, a more in-depth analysis of these factors is necessary to fully understand the risks of future species introductions into OTM.

2003–2008: Population spread

Exaiptasia pallida, once established, spread around the lake at an increasing rate, preferentially overtaking hard substrates such as mangrove roots, rocks, and fallen trees (Fig. 4). *Exaiptasia pallida* took 3 years to fully cover preferred hard substrate in the side-arm and to spread ~100 m away along the edge from the initial zone of discovery. It took only another 3 years to spread a further 500 m to fully encompass the perimeter of the lake.

The expansion of *E. pallida* 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 and Hamner 1998; e.g., Fautin and Fitt 1991, Dawson and Hamner 2005) and few predators (Hamner et al. 1982; cf. e.g., Vitousek et al. 1997, Sax and Gaines 2003). For example, known predators of *Exaiptasia*, such as nudibranchs and puffer fish (Okey et al. 2003), are absent from OTM. In addition, mutualism, as evinced in the symbiosis of *Exaiptasia*-plus-*Symbiodiniaceae* and 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). These apparently favorable conditions in OTM, coupled with the rapid asexual reproductive potential of *E. pallida*, may have contributed to the establishment and increasingly rapid expansion of the sea anemone in OTM.

Exaiptasia pallida is a widespread sea anemone, distributed worldwide in tropical and subtropical shallow waters (Grajales and Rodriguez 2014); *Exaiptasia* is suspected to be introduced throughout the Pacific (Hawaii, Japan, Mexico; Thornhill 2013). However, rapid expansions and abundances similar to OTM have been reported in only two other areas: 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 again that island characteristics—such as low diversity, absence of predators—interacting with other factors such as facilitation by a symbiont, may promote the successful invasion of *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 weather conditions of the new environment match those of the original environment (Davis 2005, Souza et al. 2011).

2009–2012: Occurrence and interactions

Following its spread around the entire lake, *Exaiptasia pallida* became one of four predominant species, though its abundance remained spatially heterogeneous and fluctuated with time. *Exaiptasia pallida* occurred disproportionately commonly on mangrove roots, suggesting negative associations should be seen mostly on native organisms also found predominantly on mangrove roots. Concomitantly, significant negative correlations were found on mangrove roots in the first three surveys, that is, when and where *E. pallida* percent cover was highest. Negative associations on mangrove roots between *E. pallida* and *C. fastigiata* (an alga), *Brachidontes* sp. 2 (a mussel), and three sponges (*Dendrilla lendenfeldi*, *Suberea* sp. 2 and encrusting sponges) on mangrove roots may indicate competition for space. The only native sponges that had a significant positive relationship with *E. pallida* were on ONS (i.e., *Speciospongia peleia*); while this may suggest a positive association, it also may simply reflect an indirect relationship tied to shared exploitation of rocky substrate. Associations with individual native species did not persist across years. Though abundance of *E. pallida* and diversity of native species were significantly different between habitats and through time, there is no significant association between the abundance of *E. pallida* and diversity of native species. The population decline of *E. pallida* between 2009–2012 suggests other environmental factors, such as changes in water temperatures and limited hard substrates, were constraining spread and limiting impacts on endemic species.

The strong population swings that we measured in *E. pallida* suggest insufficient time may have passed to infer whether the sea anemone will lead to loss of biodiversity or any other negative impact (Forrest and Taylor 2002, Davis 2003, Sax and Gaines 2003), phenomena referred to, respectively, as “extinction debt” (Sax and Gaines 2003) and “invasion debt” (Simberloff 2014). The *E. pallida* invasion in OTM is a recent event—relative to several centuries of invasions in terrestrial and other marine systems—and, in the early stages of invasion, its ultimate impact on diversity may not yet be evident (see Simberloff 2014). Whether *E. pallida* can adapt to lake conditions, establish a more consistently high abundance, and thus impact native species, may take decades to detect, as in the case of introduced nitrogen-fixing shrubs and *Sargassum* alga (Olabarria et al. 2009). Such long-term effects often are facilitated through modifying ecosystem functions and habitat alteration, processes that can occur on timescales of several decades (Olabarria et al. 2009, Simberloff 2014).

Yet, whether *Exaiptasia pallida* — a sessile, photosynthetic anemone, and not an active predator — will ever lead to any loss of biodiversity is also an open question. Case studies covering centuries of NIS introductions have shown that NIS may not lead to biodiversity loss (Mooney and Cleland 2001). 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). Currently, there is no indication that *E. pallida* is monopolizing space (c.f. Schaffelke and Hewitt 2007), and there is little evidence of exclusion (c.f. Thomas and Palmer 2015). Introduced predators and pathogens are more likely to cause extinctions than are introduced competitors (Gurevitch and Padilla 2004, Davis 2003). Thus, introduced marine predators—such as *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). But, the wide array of literature covering different timescales (decades vs. centuries), NIS species' roles (competitors vs. predators), and regions and habitats (e.g., temperate grasslands vs. tropical oceans) depicts the idiosyncrasies and complexity of invasion biology, limiting our ability to predict NIS impact in novel systems. This may be particularly so in species with nuanced ecological roles: the introduced sea anemone is autotrophic, heterotrophic, and may currently play only a small part in dynamics in OTM because other factors may affect the endemic assemblage on a larger scale (e.g., Martin et al. 2006).

Conclusion and Implications: Conservation and management of marine lakes

This case study of *E. pallida*, a sea anemone introduced into Jellyfish Lake (OTM), demonstrates that isolated marine lakes, like other islands, with low diversity and few predators, are susceptible to invasions. The rapid expansion, though aesthetically alarming, has had very little measurable impact on native diversity during these 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 alter the current native–exotic relationships in OTM (see Davis 2003, Sax and Gaines 2003, Fridley et al. 2007).

Though we found little evidence of strong negative association of *E. pallida* with native biodiversity, other case studies of NIS have described ecological and socio-economic impacts of non-indigenous species (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 rate of introductions. Tourism can play a role in the spread of non-native species (Anderson et al. 2015), and the economy of Palau is largely reliant on tourism (Yamashita 2008). With the recent increase of tourists to Palau, the risk of NIS introductions to Jellyfish Lake is increasing as tourists increased propagule pressure. Because predicting introductions of NIS and their impact is difficult (Simberloff 2014), management efforts should explicitly evaluate the risks associated with vectors and NIS introductions into this key revenue-generating

tourist site. Prevention of NIS is easier to monitor and enforce, while eradication of NIS, if at all possible, 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 could lead to alternative, unfavorable, ecological and socio-economic outcomes. At the least, the case study of the *E. pallida* 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.

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Supplementary Material

The following supplementary material accompanies this paper:

Supplementary Dataset S1. Percent cover of species per survey for each quadrat (Site no). Information regarding substrate, diversity and evenness (last 2 columns) are included.

Supplementary Documentation I. Sequencing of *Exaiptasia pallida* and its zooxanthellae

Supplementary Documentation II. PERMDISP, SIMPER, Spearman rho results

This material is available as part of the online article from <https://escholarship.org/uc/fb>

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