Conus bonus! Facilitation of Conus gastropods by invasive mangroves in Mo'orea, French Polynesia

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Background. Ecosystem engineers are organisms that modify habitats and in many cases community assemblages. Mangroves (Rhizophora stylosa) on Mo'orea displace native marsh grass habitats and affect the distribution of native and non-native gastropod species. This study aimed to determine the effect of mangrove facilitation on the expansion of geographic distribution of certain cone snail species (Conus eburneus, C. frigidus, C. leopardus, C. miliaris, and C. pulicaris).

Methods. Comparisons of temperature as well as cone snail abundance and diversity were conducted between marsh grasses, mangroves and sand flat habitats. The effects of short-term exposure to high temperatures on oxygen consumption were compared based on habitat parameters.

Results. While cone snails were absent from salt marshes, cone snails varied in abundance and diversity in mangrove and sand flat habitats. Cone snails were slightly more abundant in mangroves than paired sand flat habitats. Conus species assemblages were comparable in paired mangrove and sand flat habitats. One effect of mangrove ecosystem engineering is reduced thermal maxima and variance, which did not have a clear effect on oxygen consumption.

Discussion. Cone snails are excluded from salt marshes dominated by Paspalum vaginatum, however they are present in mangrove salt marshes. Habitat usage by cone snails is comparable between adjacent sites, suggesting that sand-specialized cone snails may receive the greatest benefit from mangrove facilitation due to proximity between possible habitats. Despite well documented effects of temperature variance on intertidal gastropods, this study did not find a direct effect of temperature variance on cone snails. This study contributes to efforts to understand effects of invasive ecosystem engineers on native community assemblages. Redistribution of species on a global scale has varied and complex implications; understanding these effects is crucial to predicting effects on biodiversity.
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Abstract  

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Introduction  

Our world is currently experiencing a period of unprecedented species redistribution (Viard, David & Darling, 2016). Globalization has rapidly allowed species to overcome biogeographic barriers and to extend their distributions beyond their native range (Mooney & Cleland, 2001). The literature on invasion biology is extensive, with much of it focusing on interactions between invasive and native species such as competition and predation (Ruiz et al., 1999; Parker, Simberloff & Lonsdale, 1999). The addition of non-native species can create more varied and complex interactions within ecosystems. For example, Crooks (2002) highlights the role of biological invaders as ecosystem engineers. Ecosystem engineers are defined as species that modify biotic and abiotic factors in their habitat (Jones, Lawton & Shachak, 1994). Invasive ecosystem engineers have varying effects, including facilitation of both native and non-native species through habitat modification (Crooks, 2002; Rodriguez, 2006). Islands provide a natural laboratory to study the complex effects of invasive organisms, since the proportional abundance...
of invasive species on island communities is generally higher than in mainland environments (Mooney & Cleland, 2001).

The invasion of some islands by mangroves is one example of an ecosystem engineer introduction (Demopoulos & Smith, 2010). The establishment of the red mangrove (*Rhizophora mangle*) in Hawai‘i typifies impacts of invasive ecosystem engineers. *Rhizophora mangle* modulates nutrient cycling and energy flow, which facilitates higher densities of introduced macroinvertebrates (Simberloff, 1990; Demopoulos & Smith, 2010). The introduction of the stilted mangrove (*Rhizophora stylosa*) to Vainahoe Bay, Mo‘orea in the 1930’s aimed to employ aerial prop roots for oyster culture (Acutt, 1995; Gershman, 1997). Despite the failure of mangrove-facilitated oyster culturing, mangroves have become established in many sites around Mo‘orea (Acutt, 1995; Gershman, 1997).

Establishment of mangrove stands on Mo‘orea is associated with changes in abiotic and biotic modifications. Healthy mangrove ecosystems can offer structure as well as reduce temperature fluctuations by providing canopy cover (Primavera, 1997; Gilma et al., 2013). Additionally, mangroves impact floral and faunal community assemblages. Kramer (1992) documented loss of habitat of *Paspalum vaginatum*, a native marsh grass, due to mangrove colonization. Mangrove stands demonstrated a disparate gastropod species assemblage from that of marsh grasses or adjacent rocky intertidal zones (Gershman, 1997). Due to high relative abundance of non-native species in mangrove stands, Gershman (1997) proposed that the invasion of *R. stylosa* facilitates the expansion of geographic distribution of non-native species.

In addition to increasing the frequency of non-native species, invasive ecosystem engineers can also create new habitat for native species. The genus *Conus*, also called cone snails, contains 500 species of predatory gastropods, 38 of which are native to Mo‘orea (Kohn, 1994; Moorea Biocode Project Database, 2016). Cone snails have diverse feeding patterns and niche specializations (Duda & Alan, 2007) and can specialize in a variety of habitats, including subtidal reef platforms, back reefs, barrier reefs, and sand flats (Kohn, 1959; Kelly-Garrick, 2010). Three of the species native to Mo‘orea have been documented in sandy habitats, and are therefore potential candidates to profit from a stilted mangrove invasion: *C. eburneus*, *C. leopardus*, and *C. pulicarius* (Kohn, 1959; Marsh, 1970; Taylor, 1986; Kelly-Garrick, 2010). Furthermore, prey specialization of native cone snails may allow for inferences into the trophic ecology of the mangrove habitat since predators are constrained by prey distribution.

Cone snail biodiversity holds direct societal value, such as the development of cone snail-derived medicines including pain and seizure treatment (Alonso et al., 2003). Terlau and Olivera (2004) estimate that there are more than 50,000 components of toxins employed by cone snails with little overlap between species, suggesting that each species is a potential source for novel pharmaceuticals. Venoms can even vary between geographic populations of the same species (Duda et al., 2009). In order to accurately determine pharmaceutical possibilities, more information about the biodiversity and ecology of cone snails is necessary.

The current study adds to the understanding of invasive ecosystem engineers by determining the effects of invasive ecosystem engineers on the distribution of native cone snails. More specifically, this study focuses on the effects of invasive *Rhizophora stylosa* on cone snail assemblages in Mo‘orea’s coastal ecosystems. The study will compare distribution of cone snails in native marsh grass habitat, invasive mangrove habitat, and adjacent sand flat habitat. This study will test whether ecosystem modulations will expand the geographic range of some, but not all, *Conus* species in Mo‘orea. Sand specialist species will gain the greatest advantage due to sand flat proximity to mangroves as well as comparable substrate. Furthermore, significant
abiotic differences between marsh grass habitats and mangrove systems will limit distribution of cone snails.

**Methods**

**Study sites**

The study was conducted on Mo’orea, a high volcanic island, surrounded by a barrier reef of conglomerate coral in the Society Islands of French Polynesia. The invasion of the stilted mangrove, *R. stylosa*, in Mo’orea provides opportunities to study the effects of invasive ecosystem engineers. *Rhizophora stylosa* is established in a number of discrete sites; five sites sampled in this study are shown in Fig. 1. Site 1 (17°29’31” S, 149°53’25” W) was located in Papeto’ai in northwestern Mo’orea; Site 2 (17°33’17” S, 149°52’44” W) and Site 3 (17°33’22” S, 149°52’30” W) were located close to Ha’apiti on the southwestern part of the island; Site 4 (17°34’18” S, 149°51’59” W) was located South of Ha’apiti; Site 5 (17°34’59” S, 149°51’9” W) was located near Vai’anae. Sites were selected by consulting maps of *R. stylosa* colonization and initial surveys (Kramer, 1992). Sites were chosen for this study because they had coastal mangroves stands as well as adjacent sand flat habitats. Sand flat habitats were a control for bordering mangrove habitat. In addition, Sites 2 and 3 contained *P. vaginatum* marshes, which were key for determining presence of cone snails in marsh grass-dominated ecosystems. Mangrove and marsh grass habitats were selected to include monospecific stands as well as 3 m of bordering sand. Sand flat habitats were selected to be parallel to shore, 10-15 m from *R. stylosa* or *P. vaginatum* stands.

**Field surveys**

Timed transects were conducted for 30 minutes in each habitat at each site parallel to shoreline, starting 10 minutes after sunset since cone snails are active at night (Kohn, 1959). Marsh grass surveys were conducted until the entire habitat had been surveyed (8 minutes at Site 2, 17 minutes at Site 3). Surveys were conducted with two surveyors, walking or snorkeling if depth permitted. Cone snails were identified and tallied by species. If a cone snail could not be identified, it was collected with metal tongs and compared to a reference of known species in Mo’orea, compiled from information in the Moorea Biocode Project (Moorea Biocode Project Database, 2016).

**Temperature comparison**

Three HOBO 64K Temperature Loggers (Onset Computer Corporation, Bourne, MA, USA) were placed in habitats of Site 2 to compare daily temperature fluctuations. Trackers were placed next to marsh grass stands, in mangrove root structures and in the sand flat habitats. Each tracker was secured with a zip tie and rope to a cinder block and buried under 3 inches of substrate. Temperature was recorded every 30 min for four days (November 4-7, 2016).

**Temperature experiments**

Temperature experiments compared the effects of diurnal maxima on cone snail metabolic rate. Twenty-seven *Conus eburneus* were collected from Site 2 and separated randomly using a random sequence generator into three treatment groups of nine each: marsh grass, mangrove and sand flat. Specimens were held in captivity for five days to acclimate to reduce systematic change from capture (Mangum and Sassaman, 1968). Snails were placed in sealed 50 ml falcon tubes for 30 min trials in water baths, as well as a blank tube. Data collected for temperature
comparisons (Fig. 2) was used to select daily maximum temperatures of each habitat for respiration trials. Each water bath was kept at the average daily maximum temperature of the habitat: 31 °C for sand flat habitat; 32 °C for mangrove habitat; and 41 °C for marsh grass habitat. Respiration was determined by measuring dissolved oxygen content in the vial at the end of the trial and comparing to blank tube. Water from falcon tubes was transferred to plates of a Loligo Microplane Reader System, minimizing air contact. Dissolved Oxygen levels were compared to initial samples of water to determine total respiration. Results were then normalized using wet mass of the snail.

Data analysis
All statistical tests were conducted using R 3.3.1 (R Core Team, 2013). An ANOVA and a Tukey’s Posthoc Test were run between marsh grass presence and control site presence to describe presence of cone snails in marsh grass ecosystems. A nested ANOVA was run to compare abundance of cone snails in mangrove ecosystems to that of sand flats, followed by a Tukey’s Posthoc Test, which tested variance by site as well as by habitat. A correlation was used to compare abundance between paired habitats of each site; a Regression Analysis, followed by a Spearman’s Rank Test were used to determine the significance of the relationship as well as the strength of the correlation. An ANOVA and a Tukey’s Posthoc Test were used to compare species diversity between each site. An ANOVA was used to compare the daily maxima by habitat, followed by a Tukey’s Posthoc Test. Coefficients of variance were calculated by dividing variance by mean values of each site. An ANOVA and a Tukey’s Posthoc Test were used to compare oxygen consumption of each treatment group.

Results
Field survey
Cone snails were present at all sand and mangrove habitats; however, none were found in any marsh grass sites. Mean snail abundance in mangrove habitats was 32.0 ± 27.5 (N=5); sandy habitat abundance was 25.4 ± 23.7 (N=5). Both were higher than abundance in marsh grass habitats (ANOVA: F=10^31 (2, 9 df); p<0.001). Figure 3 shows significant variance of cone snail abundance by site as well as by habitat (Tukey’s Post Hoc Test). Significant differences occurred between sites (Nested ANOVA: F=83 (4, 4 df); p<0.001), however certain sites shared some overlap. Figure 3 shows abundances by site and habitat and represents the significant groupings of sites. Figure 4 compares the same data, using correlation and a regression analysis.

Abundance in mangroves was correlated to adjacent sand flat habitat (Regression: F=115 (1, 3 df); r^2=0.9661; p<0.01).

As shown in Table 1, seven species of cone snails were represented in sand flat habitats; five species were represented in mangroves; no individuals were found in marsh grass ecosystems. Although more total species were found in control habitats, there is not a significant relationship between paired habitat surveys (Welch’s Two Sample t-Test: 7.4 df; p=0.73).

However, significant variation occurred between sites (Tukey’s Post Hoc Test). Figure 5 shows number of species by site as well as significant groupings. Conus eburneus was most common, accounting for 58.1% of all individuals sampled.

Temperature analysis
Temperatures, as shown by Fig. 2, fluctuated in a diurnal pattern in all habitats sampled ranging from 25 °C to 43 °C. Mean temperatures varied slightly between habitats: 28.6 °C in mangroves;
29.5 °C in sand flat habitat; 31.6 °C in marsh grasses. Coefficients of variation were more distinct: 3.6% in sand flat habitat; 15.2% in mangroves; 77.5% in marsh grasses. Average maxima of habitats were 31.0 °C in sand flat habitat, 32.0 °C in mangroves and 40.1 °C in marsh grass. Marsh habitat had significantly higher daily maxima than both marsh and mangrove ecosystems (ANOVA: F=46.41 (2, 9 df); p<0.001; p<0.001). No significant difference was detected between sand and mangrove habitats (p=0.65).

**Temperature experiments**

Respiration rates varied greatly: 2-19 ppm Oxygen/gram body mass (Fig. 6). Mean values for treatment groups were comparable: 8.5 ppm O2/g for sand treatment, 11.1 ppm O2/g for mangrove treatment and 10.5 ppm O2/g for marsh treatment. No significant difference was detected between treatment groups (ANOVA: F=1.64 (2, 24 df); p=0.22). No snails died during temperature trials.

**Discussion**

**Gastropod Distribution**

Previous comparative studies of gastropod assemblages in mangrove and marsh grass communities of Mo’orea did not include the genus *Conus*, possibly due to diurnal sampling (Gershman, 1997). Nocturnal hunting makes them difficult to identify during the day, therefore diurnal surveys might not fully account for cone snail presence. The field survey expands on previous studies by demonstrating the presence of five cone snail species in mangrove habitats (Kelly-Garrick, 2010). Cone snail presence in mangroves demonstrates their ability to inhabit mangrove-engineered ecosystems. Mangrove invasion of salt marshes facilitates cone snail’s expansion since mangroves convert habitat that was previously occupied by marsh grass (Kramer, 1992).

Abundance is slightly higher in mangrove habitats than adjacent sand flats. This trend may be due to reduced predation in mangrove habitats. For example, shrimp experience lower predatory pressure due to shelter offered by mangrove prop roots (Primavera, 1997). *Conus* species assemblages (Table 1) in mangroves are comparable to those found in adjacent sand flat habitats. All five species of cone snail represented in the mangrove surveys (*C. eburneus*, *C. pulicarius*, *C. miliaris*, *C. leopardus* and *C. frigidus*) were also found in control habitats. Three of the species present in mangroves (*C. eburneus*, *C. pulicarius*, and *C. miliaris*) have been documented in sandy habitats before (Kohn, 1959; Marsh, 1970; Taylor, 1986; Kelly-Garrick, 2010). Therefore, it appears that most of the cone snail species facilitated by mangroves are present in sandy habitats.

The most common species in both mangrove and sand flat habitats was *C. eburneus*, followed by *C. pulicarius* and *C. miliaris*. These three species are vermivorous, and feed chiefly on polychaete worms (Marsh, 1970). Therefore, polychaete worms must be present in or near mangrove habitats. *Conus leopardus* was present in lower numbers than other identified sand specialists. However, its density and presence in sand flat habitats is consistent with previous studies (Kelly-Garrick, 2010). *Conus leopardus* feeds on *Ptychodera flava*, an enteropneust found throughout the Indo-Pacific (Kohn, 1959). Therefore, *C. leopardus* hunting in mangrove habitats suggest the presence of enteropneusts. *Conus frigidus* preys on polychaetes, but has previously been recorded in limestone reefs (Kohn & Nybakken, 1975). Presence of *C. frigidus* in sand flats and mangrove habitats may reflect generalist behaviors on Mo’orea.
Two species of cone snails were found in sand flat habitats, but were absent from paired mangroves. *Conus textile* and *C. flavidus* were recorded in low numbers at Site 2 and *C. flavidus* was recorded in sand flats at Site 4. Therefore, sampling may have not have measured the low abundance in mangrove habitats. Alternatively, it is possible that these two species are excluded from mangrove habitats either by prey presence or for other physiological reasons. More extensive observations would be required to determine the presence of *C. textile* and *C. flavidus* in mangrove habitats.

**Temperature fluctuations**

According to Jones, Lawton, and Shachak (1994), an ecosystem engineer modifies resource availability by physically altering biotic and abiotic resources. Moderating temperature fluctuations is one way that mangroves engineer habitat on Mo’orea’s coastline. Temperature, as shown in Fig. 2, had higher coefficients of variance as well as significantly higher daily maxima in marsh grass habitats compared to both mangroves and sand flats. Intertidal temperatures can vary rapidly and can have effects on the physiology of intertidal invertebrates, including “heat comas” and death if thermal ranges are exceeded (Broekhuysen, 1940; Newell, 1969; Clarke, Mill, & Grahame, 2000; Davenport & Davenport, 2007). Reduced variance and daily maxima may make mangrove habitat more hospitable to invertebrates, including cone snails and prey species, such as polychaetes and enteropneusts. Therefore, range of cone snails may be directly limited by thermal fluctuations, or indirectly by exclusion of prey species.

**Temperature experiments**

High temperatures are known to limit intertidal gastropod distribution, reduce activity drastically, and even kill snails (Broekhuysen, 1940; Davenport & Davenport, 2007). Figure 6 shows oxygen consumption, which did not demonstrate significantly reduced activity in high temperature trials. There was no death during the temperature trials. These results may be due to air exposure while conducting measurements. Alternatively, it is possible that intertidal cone snails tolerate wide temperature ranges, especially due to minimal diurnal activity. Diurnal rest may be comparable to ‘heat comas’ described by Clarke, Mill and Grahame (2000) in other gastropod species. However, trials did not account for the duration of diurnal temperature fluctuations. Trials lasted thirty minutes and may have ignored effects of long term exposure. Conducting two hour trials in 40˚C conditions, Broekhuysen (1940) found 100% mortality rates of five of the six South African intertidal gastropod species he studied. Although these values cannot be directly compared, this demonstrates the effects of lasting heat exposure on a variety of gastropods. Longer exposures at high temperature may demonstrate higher mortality rates in cone snail species of Mo’orea.

**Conclusion**

Mangroves altered salt marsh community assemblages and facilitated the colonization of cone snail species. The species of cone snail that colonize mangrove habitats are: *C. eburneus*, *C. pulicarius*, *C. miliaris*, *C. leopardus*, and *C. frigidus*. These species are also present in adjacent sand flat habitats. However, cone snails are not present in marsh grass ecosystems. Marsh grass habitats have significantly higher temperatures and higher coefficients of variance. Thermal parameters may make marsh grass ecosystems inhospitable to cone snail species or prey species. Future studies should fully assess the thermal effects on distribution to determine factors limiting distribution of cone snails in marsh grass habitats. Additional studies should study the effects of
long-term heat exposure on cone snail survivorship. Additionally, surveys should compare the
presence of prey species— polychaetes and enteropneusts— in marsh grass habitats to that of
mangrove habitats to determine the effects of ecosystem engineers by trophic level.

This study addresses the role of invasive *R. stylosa* in habitat and *Conus* gastropod
community assemblage modification in Mo’orea. These conclusions are valuable for
understanding trophic ecology in mangrove habitats. More globally, this is a complex
relationship of an invasive species facilitating geographic expansion of a native species. Invasive
ecosystem engineers can have indirect, yet drastic, effects on the distribution of another
species. Modification of abiotic factors can alter standard nutrient flow, temperature, and other
factors. This can expand or reduce habitat for native species. Additionally, it can alter biotic
factors by removing prey species, or increasing predation. Therefore, evaluation of invasive
species impacts must include many biotic and abiotic variables that may indirectly affect
biodiversity.

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Stauffer-Olsen; and my classmates who were editors, research assistants and friends.


Figure 1 (on next page)

Map of Mo’orea and study sites.

Site 1: 17°29′31″ S, 149°53′25″ W; Site 2: 17°33′17″ S, 149°52′44″ W; Site 3: 17°33′22″ S, 149°52′30″ W; Site 4: 17°34′18″ S, 149°51′59″ W; Site 5: 17°34′59″ S, 149°51′9″ W
Site 1

Site 2

Site 3

Site 4

Site 5
Figure 2 (on next page)

Temperature with respect to time in sand flat, mangrove, and marsh habitats.
Abundance of cone snails by site and habitat.

Letters dictate significant groups.
Correlation of mangrove abundance to control abundance.

Mangroves correlate closely to paired sand flat sites, but have slightly higher abundance (y=1.146x+3.682; r²=0.9661; p<0.01).
Abundance of *Conus* species in each mangrove and sand habitats.
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Figure 5 (on next page)

Species diversity by site.

Letters show significant groups.
**Figure 6** (on next page)

Respiration rates by treatment