Manuscript 1 draft

Introduction

Habitat subsidies can strongly shape the structure and function of recipient ecosystems [(Helfield and Naiman, 2001, Jefferies, 2000, Menge et al., 2003, Nakano et al., 1999, Palumbi, 2003, Polis and Hurd, 1996, Spiller et al., 2010)](https://www.zotero.org/google-docs/?tVsRfj). Such subsidies will likely change (quality and quantity) as humans alter the composition of species in donor ecosystems via species introductions and range shifts associated with climate change. Ecological theory (e.g. Enemy Release and Home-Field Advantage Hypotheses) predicts that these novel subsidies will 1) be underutilized by species in naïve recipient ecosystems, 2) suppress performance of naïve species, and 3) shift grazing onto native prey. However, we lack experimental tests about the consequences of human-mediated alterations of donor ecosystems on naïve recipient ecosystems. Thus, we used mesocosm experiments to compare the preference and performance of naïve rocky intertidal grazers when fed native or invasive seaweeds (or a mixture of both), and the impact of this shift on native benthic seaweed communities.

Ecosystems are connected through fluxes of nutrients, organic particles, and larvae from donor systems that can alter species abundances and interactions in recipient food webs [(Gratton et al., 2008, Greig et al., 2012, Nakano et al., 1999, Polis and Hurd, 1996)](https://www.zotero.org/google-docs/?wvYtc7). These subsidies can change the densities of key animal species and increase rates of ecological processes such as competition and predation in recipient ecosystems [(Greig et al., 2012, Palumbi, 2003)](https://www.zotero.org/google-docs/?wRkCyv), and in extreme cases, can lead to trophic cascades [(Jefferies, 2000, Polis et al., 1997, Polis and Hurd, 1996)](https://www.zotero.org/google-docs/?mn715V). It is well established that marine phytodetritus, dislodged seagrasses, and seaweeds washed ashore (collectively termed “wrack”) have significant ecological consequences to nearshore and shore habitats [(Bustamante et al., 1995, Duggins et al., 1989, Orr et al., 2005)](https://www.zotero.org/google-docs/?25X4OH).

For example, wrack on Bahamian Island coastlines temporarily shift predator behavior [(Kenny et al., 2017)](https://www.zotero.org/google-docs/?YvsLXJ), increase predator densities [(Piovia-Scott et al., 2013)](https://www.zotero.org/google-docs/?aQ3N2R), and ultimately result in herbivore-mediated trophic cascade on some plants [(Piovia‐Scott et al., 2019)](https://www.zotero.org/google-docs/?Ws0DIk).

The impact from subsidies depends on the frequency, magnitude, and quality of the subsidy [(Yang et al., 2010)](https://www.zotero.org/google-docs/?6PvQXk). A combination of anthropogenic perturbations such as nutrient depositions, overharvesting, altered predator guilds, and climate change are altering natural ecosystems [(Falkowski et al., 2000, Greig et al., 2012, Worm et al., 2006)](https://www.zotero.org/google-docs/?WGalYb), thus, altering the quality and quantity of subsidies between ecosystems. Moreover, climate change is expected to increase the ranges of some species, and human activities increasingly move non-indigenous species beyond their normal range [(Rilov and Crooks, 2009, Ruiz et al., 1997)](https://www.zotero.org/google-docs/?ygqfQW). Because various processes drive invasions and extinctions (e.g., ballast water transport and over harvesting, respectively) the types of species gained or lost may differ [(Byrnes et al., 2007)](https://www.zotero.org/google-docs/?9UPxiw). Thus, changes in wrack composition due to non-indigenous species introductions may have species-specific impacts on recipient native communities that depend on that subsidy [(Rodil et al., 2008)](https://www.zotero.org/google-docs/?FOSf5P)**.**

Many beaches and rocky intertidal shores worldwide receive large inputs of wrack from offshore donor systems. However, few studies have examined these impacts experimentally, especially in the context of human-mediated alterations to donor systems on naïve recipient ecosystems [(Rodil et al., 2008, Suárez-Jiménez et al., 2017)](https://www.zotero.org/google-docs/?T2GNua). To fully understand the consequences of introduced species, it is crucial to examine their impacts on the communities that receive the non-indigenous species as a source of food. This is especially important when recipient communities include sensitive and endangered species that rely on wrack. For example, the recent growing invasion of Devilweed (*Sargassum horneri*) in kelp forests[(Marks et al., 2015)](https://www.zotero.org/google-docs/?0ijcyF) may impact wrack-associated communities, including endangered Black abalone (*Haliotis cracherodii*) on rocky shores of Southern California and the Channel Islands .

The Enemy Release Hypothesis predicts that the liberation of non-indigenous species from their natural predators makes them more successful invaders of novel ecosystems. Further, the Home-Field advantage predicts that decomposer communities are specialized to consume foods they frequently encounter. Thus, we hypothesize that novel subsidies will 1) be underutilized by species in naïve recipient ecosystems, 2) suppress performance of species in naïve recipient ecosystems, and 3) shift grazing onto native prey in naïve recipient ecosystems.

To test these hypotheses, we offered an assemblage of rocky intertidal grazers, Striped shore crabs (*Pachygrapsus crassipes*), Blue banded hermit crabs (*Pagurus samuelis*), and Black turban snails (*Tegula funebralis*) a choice between native California Giant kelp (*Macrocystis pyrifera*) and non-native Devilweed (*Sargassum horneri)*. Giant kelp is ubiquitous throughout southern California and accounts for a large part of the wrack. Due to the recent invasion, Devilweed is increasingly found in the wrack on the mainland coast and on the California Channel Islands and interactions with naïve, native grazers will likely increase as the expansion of Devilweed continues. To test how a diet including Devilweed impacts the performance of grazers, we fed snails diets that include Devilweed and measured soft tissue growth and righting times (see methods, and supplemental material). Lastly, we offered an assemblage of grazers agar-based foods made from native benthic seaweeds and either native kelp or non-native Devilweed to determine if consumption is shifted onto native seaweeds in the presence of a novel seaweed.

Methods

*Study sites and organisms*

Striped shore crabs , Blue banded hermit crabs, and Black turban snails are abundant grazers along rocky shores of southern California and the Channel Islands [(Aquilino et al., 2012, Barry and Ehret, 1993, Abbott and Haderlie, 1981)](https://www.zotero.org/google-docs/?Dl2goz). Black abalone were historically important grazers here prior to declines related to disease and overharvesting. Currently, Black abalone densities have started to recover on some of the Channel Islands. We included these organisms in the study as they represent a typical assemblage of rocky intertidal grazers in areas experiencing the invasion of Devilweed.

*Surveys*

To document the prevalence of Devilweed in wrack in this region, we surveyed large wrack piles at five sites on San Nicolas Island. We haphazardly selected piles of wrack longer than 1 m and calculated the proportion of each pile’s surface that consisted of Devilweed. For each pile, we measured the longest length and width to obtain a total surface area using the formula for ellipses (*A=πab*) where A is the total area calculated by multiplying length (a), width (b), and pi. To determine the proportion of surface area consisting of Devilweed*,* we visually scanned the piles without disturbing them, and measured the maximum length and width of any Devilweed individuals found on the surface. The proportion of Devilweed was calculated by dividing the total area of devil weed by the total area of each wrack pile.

*Feeding Choice Assays*

To understand how shifting wrack composition might affect the behavior of rocky shore grazers, we offered grazers a feeding choice of Giant kelp (hereafter referred to as kelp) and Devilweed. Kelp was collected as fresh wrack from Ocean Beach (GPS) and Devilweed was collected by snorkel from the jetty at Mission Bay in San Diego (32°76’189.1"N, -117°24’550.3"W). Once collected, the seaweeds were transported to San Diego State University’s Coastal and Marine Institute Laboratory (CMIL) and kept in recirculating tanks at 14°C for three days before the assays. Separate feeding choice assays were conducted with each of the four grazer species. Striped shore crabs, Blue banded hermit crabs, and Black turban snails were collected from Sunset Cliffs Natural Park (32°72'627.3"N, -117°25'144.0"W) and transported to CMIL. These grazers were acclimated in aquaria with flow-through, ambient temperature water and fed *Ulva spp.* for three days, and then starved for 2 days. Feeding the grazers *Ulva spp.* reduced between-individual variation in feeding history and starvation motivated the grazers to feed during the assays. With the exception Black abalone, all assays were conducted simultaneously (June 2019). The Black abaloneassay was conducted later in the summer (August 2019) at the NOAA Southwest Fisheries Center.

We conducted feeding choice assays by placing 3 individual Black turban snailsor Blue banded hermit crabs in the center of plastic containers (190x160x110mm, n=14). In each container, we also placed a similar biomass of Devilweedand kelp, anchoredwith binder clips. The Striped shore crabassay was conducted using only 1 individual per container to prevent overcrowding and intraspecific competition (n=15). Black abalone were also housed individually in 280x230x180mm mesh-lined, PVC-framed cages with a Plexiglas floor. All replicates were paired with no-grazer controls to account for changes in mass unrelated to consumption [(Dolecal and Long, 2013)](https://www.zotero.org/google-docs/?dg6iCH). Individual replicates were stopped when grazers consumed  approximately 75% of either seaweed or when the experiment reached 6 days. All remaining seaweed and seaweed fragments were identified, blotted dry and weighed. We adjusted for autogenic growth using Equation 1, Ti (Cf /Ci) – Tf, where Ti and Tf represent the seaweed masses of the experimental treatments and Cf and Ci represent the seaweed masses in control treatments before (i) and after (f) the assay [(Dolecal and Long, 2013, Sotka and Hay, 2002)](https://www.zotero.org/google-docs/?FkRTca).

*Performance Assays*

To understand how shifting wrack populations might affect the performance of rocky shore grazers, we fed two grazer species (Black turban snails and Red abalone (*Haliotis rufescens*)) either kelp or Devilweed. We used Red abalone as a proxy for Black abalone because 1) they are congeners whose performance might be affected similarly by Devilweed as their growth rates depend on water temperature, oxygen supply, food availability, and water circulation rates [(Venter et al., 2018)](https://www.zotero.org/google-docs/?ksagZ4) 2) most captive Black abalone are mature adults that might not readily reveal impacts on performance as measured by growth. We recognize that Black abalone and Red abalone are different in their ecology, however, feeding trials in aquaria show that brown seaweeds are generally important components of *Halitidae* diets in the northern hemisphere [(Leighton and Boolootian, 1963, Nelson et al., 2002, Winter and Estes, 1992)](https://www.zotero.org/google-docs/?zR4h1u).

Grazers were kept individually in plastic containers (190x160x110mm) with mesh (1mm) tops and mesh covered holes on two sides, to allow increased water flow. All containers were completely submerged in tanks with 14°C recirculating water and aerators. Grazers were offered one of four seaweed diet treatments (kelp*,* Devilweed*,* mixture of kelp and Devilweed, or starved, n=20). Biomass of seaweed was not standardized, however, all grazers were given enough seaweed tissue to not be food limited. We included parts of the entire thalli (i.e., stipes, blades, and pneumatocysts) to account for any differences in tissue type.

We collected Black turban snails from Sunset Cliffs Natural Park (Shell length = 6-10mm. All organisms were transported to CMIL and placed in plastic containers (1190x160x110mm) with mesh (1mm) covers as described above. All containers were completely submerged in tanks with 14°C recirculating water and aerators. We used lab-reared, juvenile Red abalone of the same cohort (May, 2017), donated by NOAA Southwest Fisheries (La Jolla, California). Because juvenile abalone have a greater capacity to grow than adults, we selected the smallest individuals for this experiment (45-60mm in length).

We assessed diet-related impacts on performance by calculating the change in dry soft tissue mass. Final dry tissue mass was calculated by weighing this tissue after dissecting it from the shell and drying the tissue at ### C for ### hours/days. To estimate initial dry tissue mass, we subsampled 20 individuals from each grazer species at the start of the experiment and calculated a regression of shell length to dry soft tissue mass. This regression was then used to estimate soft tissue biomass, non-lethally, based on shell length measurements.

In addition to impacts on growth, we used these same animals to assess impacts on defensive behaviors. Specifically, we measured the amount of time abalone needed to right themselves [(Baldwin et al., 2007, Lachambre et al., 2017)](https://www.zotero.org/google-docs/?c7rCjJ). Righting tests involved placing abalone, dorsal side down, in a 14°C water tank, and timing how long it took them to use their foot muscles to completely flip their orientation. The tests were conducted until abalone corrected their orientation or timed out at 4 minutes.

*Community Grazing Assay*

To understand if shifting wrack populations (from kelp to Devilweed) causes intertidal grazers to shift consumption onto native benthic seaweed, we offered an assemblage of intertidal grazers a choice of native benthic seaweed in the presence of either kelp or Devilweed. We used an assemblage of grazers and seaweed proportional to densities of populations at Sunset Cliffs Natural Park (Black turban snails n=3, Blue banded hermit crabs n=6, Striped shore crabs n=1). We offered grazers a choice between Sea lettuce *Ulva spp.,* brown rockweed (*Silvetia compressa*)*,* and red benthic seaweed (*Centroceras clavulatum*)and either Devilweed or kelp*.*

For this assay, we offered grazers agar-based foods made from freeze-dried, homogenized seaweed. We adopted this procedure for logistical reasons related to the timing of seaweed collections and starting assays. We note that this process maintains chemical traits while removing morphological traits that may also impact palatability.  Following methods adopted from previous studies [(Bolser and Hay, 1996, Dolecal and Long, 2013, Thornber et al., 2008)](https://www.zotero.org/google-docs/?ak5UqY) we mixed homogenized seaweed (5.6% weight:volume) into a heated agar solution (2.0% w:v). The solution was poured into polypropylene petri dishes (50 mm) and allowed to cool for ~30 min. Once ready, the agar-foods were blotted dry and weighed before being offered to grazers.

Based on previous experiments showing negligible autogenic growth in agar-based foods and due to tank space limitations, we reduced the number of controls to one control for every two experimental treatments. We used thermoplastic adhesive to secure the petri dishes containing NBS and either kelpor Devilweed in random order along the length of 330 x 190 x 108 mm containers with mesh (1mm) covered tops (n=20, and n=10 controls). The grazers were placed on the opposite side of the container and allowed to feed until at least half of any food was consumed. At this point, all petri dishes were removed from the replicate, blotted dry and weighed. We calculated changes in mass using the autogenic growth formula with the average correction factor from controls.

*Statistical Analyses*

*Feeding Choice Assays*

All statistical analyses were conducted using R Studio (V. 1.4.1717). Necessary assumptions were met for statistical analyses and post-hoc tests were conducted as needed. For feeding choice assays, we compared changes in seaweed mass, corrected for autogenic growth, using Paired t-tests for each grazer species.

*Performance Assays*

We estimated initial dry soft tissue masses using linear regression models. We analyzed grazer species independently by using Analysis of Covariance (ANCOVA) to test the effect of all diet treatments including seaweeds (i.e. all but “starved” treatment) on growth. We compared final dry masses of grazers with initial dry mass as a covariate. A Tukey's post-hoc test was used to determine treatment differences.

*Community feeding choice assay*

To test if including Devilweed

To test if overall consumption differed between treatments, we pooled all seaweed, including Devilweedand kelpand compared total consumption using a 2-sample t-test.  To test if consumption of native benthic seaweed changes when offered with either invasive Devilweedor native kelp*,* we pooled consumption of all of the native benthic seaweed as a response variable and compared total consumption without the wrack treatments. Finally to understand if consumption shifted onto any one native benthic alga, we used Analysis of Variance to compare consumption of all seaweed by species with kelpandDevilweedas “wrack species” in the analysis.

*Results*

*Survey*

*Preference*

Grazers displayed species-specific preferences for kelp andDevilweed. Black abalone and Black turban snails preferred kelp (t=4.5053, p<0.001 and t= 5.7152, p<0.001, respectively). In contrast, striped shore crabs did not differentiate between the two wrack species (t=0.0739, p=0.9422) and hermit crabs showed a preference for Devilweed (t=-3.7593, p=0.002385).

*Performance*

We accounted for differences in starting masses of individuals using estimated initial dry mass calculated from shell lengths.The relationship between maximum shell length and dry tissue mass was strong and linear for both Black turban snails (R2 = 0.89, F(1,18)=148.20, p<0.001)and Red abalone(R2 = 0.89, F(1,18)=160.71, p<0.001)*,* thus, estimated dry mass was included as a covariate in the analysis. Diet influenced final dry tissue mass for both Red abalone (ANCOVA: F(2,56)=4.606, p=0.01) and Red abalone*.* (ANCOVA: F2,56= 3.696, p=0.03).

Consistent with our choice feeding experiments, Black abalone fed lower preference Devilweed grew less than conspecifics fed kelp. Surprisingly, turban snails that also preferred to feed on kelp actually grew less on kelp diets relative to Devilweed.

Although previous works suggest that a mixed diet can lead to higher performance (e.g. growth or reproductive output) [(Aquilino et al., 2012, Worm et al., 2006)](https://www.zotero.org/google-docs/?6u8iPW), we did not find evidence that a mixed diet resulted in significantly higher performing individuals. Surprisingly, there was no difference in performance of Black turban snail individuals in Devilweedand mixed diet treatments while those offered only kelpexhibited lowest performance (Tukeys HSD). In contrast, a diet of kelpresulted in the highest performing Red abaloneindividuals and those offered only Devilweedexhibited the lowest performance (Tukey’s HSD).

Additionally, we saw an overall treatment effect in abalone righting tests (ANOVA), however, there was no significant difference in righting times between diet treatments. Regardless, it should be noted that righting times of individuals on kelpdiets decreased while righting times for those in Devilweed and mixed diets increased after treatment (Fig. #).

*Community feeding choice assay*

When offered a

When grazers were offered a choice between native benthic seaweeds and either Devilweed or Kelp, consumption of Devilweed was significantly lower than kelp. Consumption of S. compressa was higher in Devilweed treatments but there was no significant difference in consumption of *Ulva Spp.* and *C. clavulatum*  (Nested ANOVA: F(4,152)= 99.216, p<0.001, Tukeys HSD).

Discussion

Main points

1 impact of invasive is species specific

2 may greatly affect already vulnerable species

3 preference and performance not always correlated

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