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Predatory regulation of Tegula grazing activity in giant kelp forests

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# Predatory regulation of *Tegula* grazing activity in giant kelp forests

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# ABSTRACT

Understanding the effects of predator-induced "top-down" grazing regulation is critical to effective management of subtidal rocky reefs along the California coast, where macroalgae such as the giant kelp, *Macrocystis pyrifera,* are essential to ecosystem function (Dayton 1985, Graham 2004, Falkenberg et al. 2012). Previous studies in California have documented significant correlations between fishery-reduced predator densities, increased herbivore (i.e., grazer) abundance, and subsequent decreases in macroalgal cover, suggesting that predator removal has the potential to drive kelp forest decline by disrupting grazing regulation of benthic herbivores (Lafferty et al. 2004, Hughes et al. 2013). Thus, as coastal predatory assemblages are increasingly altered by fishing pressure and other anthropogenic stressors, identifying and preserving the essential components of top-down regulation is becoming ever more urgent (Tegner and Dayton 2000. Steneck et al. 2002).

Existing research in California has focused almost exclusively on urchins, even though recent trophic interaction models identify mid-sized gastropods (e.g., *Tegula spp.)* as a critical and overlooked source of macroalgal consumption whose relative impact will likely increase as fishery-targeted grazers (i.e., urchins, larger invertebrates) are removed from the ecosystem (Sala and Graham 2002). Like urchins, subtidal *Tegula* are voracious and potentially destructive grazers in kelp forests (e.g., Chess 1983, Steinberg et al. 1995, Van Alstyne et al. 1999), and macroalgal preferences of these species appear to be driven by the presence of benthic predators (primarily sea stars). Top-down regulation of these grazers may also reflect a strong non-consumptive (i.e., trait-mediated) component (Watanabe 1984a; 1984b). Thus, evaluating the extent of top-down regulation for *Tegula spp.* could provide critical insight into the processes influencing the abundance of *M. pyrifera* off California, which have been insufficiently

explained by urchin-based grazing models that typically consider only the direct, consumptive impacts of predation (Babcock et al. 2010, Guenther et al. 2012).

Experimental field evaluations of behavioral, trait-mediated predator-grazer interactions have rarely been attempted in marine subtidal systems due to logistical constraints associated with monitoring/maintaining long-term predator exposure treatments. However, the 2013 outbreak of sea star wasting disease (SSWD) along the California coast presented a unique opportunity to conduct a natural experiment quantifying *Tegula* regulation by predatory sea stars on an ecologically relevant, reef-wide scale not otherwise achievable with conventional manipulative studies. Unlike experimental predator manipulations that are logistically limited in duration and scope, often precluding direct extrapolation of results to natural reefs, disease-induced removal of both main *Tegula* predators, the sea stars *Pisaster spp.* and *Pycnopodia helianthoides* persisted on many central California reefs for months-years following initial SSWD outbreak. Because the spatial distribution of disease occurrence was regionally widespread but locally patchy, I was able to use a paired experimental design to compare *Tegula* macroalgal association, dietary preference, and life history characteristics at reefs with severe disease outbreaks (i.e., SS- sites; complete or near predatory sea star absence) vs. low-moderate disease severity reefs (i.e., SS+ sites), where predatory sea stars retained some degree of local presence. This provided a rare opportunity to examine the long-term impacts of large-scale predator removal on *Tegula* grazing behavior and population dynamics in an ecologically realistic field setting.

With this in mind, the overarching goal of my proposed research was to characterize predator-prey interactions and evaluate the ecological significance of top-down regulation for *Tegula* species in giant kelp forests. These largely understudied grazers could increase our understanding of the ecological interactions influencing kelp forest productivity and stability off the California coast (e.g., Babcock et al. 2010, Guenther et al. 2012). Through a series of natural field experiments and subsequent modeling approaches developed based on my empirical research results, I attempt here to address the following research questions: (1) do *Tegula* spp. alter their grazing behavior, dietary preferences, and/or algal utilization patterns in the presence of predatory sea stars?, (2) does long-term predator exposure history influence morphometric growth or energy allocation patterns (e.g., reproductive investment) in subtidal *Tegula* at a local population level, and are potential responses consistent across the three *Tegula* species commonly found in kelp forests throughout central California?, and (3) what are the potential ecosystem-level impacts of predatory sea star removal on *Tegula* population dynamics and productivity and stability of lower trophic levels in kelp forest food webs?

# CHAPTER 1

# Wasted sea stars and hungry snails drive a behaviorally mediated trophic cascade in giant kelp forests

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# ABSTRACT

In central California kelp forests, sea stars represent the primary predator for subtidal turban snails, *Tegula* spp*.,* a highly abundant but largely understudied macroalgal grazer group in the region. Unlike urchins, regulatory pathways affecting snail grazing activity have not been well characterized in field settings, but if predator-driven effects are substantial, widespread sea star mortality could drive cascading regional losses of macroalgal habitats. Here, we employed the outbreak of sea star wasting disease as a unique natural experiment to evaluate potential top-down regulation of *Tegula* spp. in Monterey, Big Sur, and San Luis Obispo, California, USA. Utilizing a paired sampling design within each region, we surveyed one reef where local disease impacts were severe, removing all predatory sea stars (SS-), and a nearby reef where some predatory sea stars remained (SS+). This approach allowed us to evaluate both short-term responses in snail grazing behavior and long-term dietary and life history consequences. Despite very low predator densities at SS+ sites relative to historical baselines (0.05-0.20/m$^2$), visual surveys revealed distinct shifts in macroalgal association when sea stars were removed. Approximately 90% of snails at SS+ sites were observed on *M. pyifera,* which provides vertical refuge but low nutritional value, while 47-52% of snails at SS- sites were observed on benthic red algae, which is highly nutritious but offers limited shelter. Dietary $\delta^{13}C/\delta^{15}N$ stable isotope analysis reflected long-term shifts in snail macroalgal consumption that were consistent with observed grazing behavior, and random *Tegula* samples from SS- sites also displayed significantly larger gonads and greater numbers of newly recruited individuals compared to SS+ samples, suggesting that increased access to benthic food sources may enhance local reproductive investment. Results here indicate that predatory sea stars play a key role in regulating *Tegula* population dynamics through grazing activity, with substantial implications for local kelp forest habitats; field surveys in all three study regions here revealed consistent differences in relative macroalgal abundance between paired SS+/- sites, suggesting strong potential for a disease-induced trophic cascade driven primarily by behavioral modification rather than density-mediated effects.

***Key Words:*** *trophic cascade, TMII, kelp forest, Tegula, predatory regulation, grazer release, sea star wasting disease, Pisaster, natural experiment, stable isotopes, grazing behavior*

## INTRODUCTION

The relative importance of top-down vs. bottom-up effects in structuring coastal marine ecosystems has been widely debated over several decades (e.g., Menge 1992, Shears et al. 2008). However, recent meta-analyses of experimental results across a range of terrestrial and aquatic environments have increasingly identified predator-mediated trophic cascades as the primary mechanism determining community structure and biomass distribution in most systems examined (Borer et al. 2006, Heck and Valentine 2007), with the strongest cascading effects consistently observed in benthic marine food webs (Shurin et al. 2002, Borer et al. 2005, Burkepile and Hay 2006). In a number of aquatic studies, both predator presence and nutrient addition were identified as significant drivers of enhanced algal biomass, yet the ecosystem impacts of these processes were highly asymmetrical in that only predator-driven effects had potential to induce cascading biomass changes across multiple trophic levels (Borer et al. 2006). This suggests that the ecological importance of top-down, predator-mediated regulation in maintaining benthic production within coastal marine systems may be substantial (Heck and Valentine 2007).

To date, the majority of manipulative experiments evaluating predator-mediated trophic cascades in marine food webs have focused on intertidal organisms (reviewed in Borer et al. 2005, Heck and Valentine 2007). However, conclusions derived from these studies may also be particularly critical to the understanding and effective management of temperate subtidal systems, where macroalgae such as *Macrocystis pyrifera* (giant kelp)) often serve as foundation species, providing critical biotic habitat for coastal fishes and invertebrates in addition to primary production (Dayton 1985, Falkenberg et al. 2012). Multiple observational field studies in rocky reef systems throughout the northeastern Pacific have documented significant correlations between fishery-driven predator removal, increased abundance of sea urchin grazers, and subsequent declines in *M. pyrifera,* which represents the dominant habitat-forming macrophyte in the region (Cowen 1983, Dean et al. 1984, Tegner and Dayton 2000, Lafferty 2004). Similar but opposite directional effects have also been observed following the establishment of marine protected areas (MPAs), where enhanced predator abundance is often followed by lower herbivore densities and increased vegetative cover (Behrens and Lafferty 2004, Guidetti 2006, Hughes et al. 2013). Although largely correlative, these studies provide strong evidence that removal of predators from coastal food webs has potential to induce catastrophic cascading effects on important foundation species by disrupting grazing regulation of benthic herbivores (Tegner and Dayton 2000, Shears et al. 2008). If so, identifying and preserving essential components of top-down regulation in temperate, algal-dominated systems such as kelp forests will become increasingly important as coastal predatory assemblages worldwide are rapidly altered by fishing pressure and other anthropogenic stressors (Jackson et al. 2001).

Existing research on rocky reef-kelp forest food webs has focused primarily on density-mediated indirect interactions (DMIIs), in which predators limit macroalgal grazing pressure by consuming benthic herbivores and reducing the number of grazers on a reef (Werner and Peacor 2003, Schmitz et al. 2004). In subtidal field settings where experimental manipulations of predators may not be logistically feasible, these DMIIs can still be inferred by surveying the relative abundance of herbivores and macroalgae at study sites with variable predator presence, typically utilizing either MPA boundaries or known gradients in fishing pressure (Guidetti and Sala 2007, Babcock et al. 2010, Altieri et al. 2012, summarized in Shears et al. 2008). Urchins have been widely identified as the dominant grazer group across a number of temperate marine systems (Tegner and Dayton 2000), and as a result, most studies evaluating DMIIs in kelp forests focus almost exclusively on urchin-specific predators, which tend to be larger-bodied, commercially targeted species (finfish, spiny lobsters) for which fishing pressure is a reasonable proxy for predator presence (Kawamata and Taino 2021, Norderhaug et al. 2021). However, a handful of existing field experiments conducted in kelp forests and seagrass beds have also demonstrated that predation by microcarnivorous fishes on amphipods and other small mesograzers can significantly decrease local grazing impacts on primary producers (Davenport and Anderson 2007, Lewis and Anderson 2012). One recent experimental study conducted in subtidal stands of *Egregia menziesii* (feather-boa kelp) found that the mere presence of a common predatory fish significantly altered limpet foraging activity and reduced subsequent kelp frond loss through a behaviorally mediated trophic cascade (Haggerty et al. 2018). Because ecosystem impacts of these smaller mesopredatory species cannot be effectively evaluated across gradients in fishery activity, it is likely that some less obvious but ecologically critical regulatory pathways maintaining macroalgal production within temperate kelp forests may be overlooked using a solely observational approach.

Apart from a few notable examples (Byrnes et al. 2006, Haggerty et al. 2018), existing research has rarely considered potential non-consumptive effects of predator presence in regulating grazer foraging behavior and macroalgal loss, even though experimental evidence from intertidal and freshwater food webs has increasingly emphasized the prevalence of these trait-mediated indirect interactions (TMIIs) driving behavioral trophic cascades in other aquatic systems (Schmitz et al. 2004, Preisser et al. 2005, reviewed by Werner and Peacor 2003). Unlike DMIIs, threat cues from a single predator in TMIIs can theoretically elicit a behavioral “fear” response from all grazers present within a given detection radius, effectively decoupling suppression of grazing activity from predator abundance. This may have important ecological implications for the stability of top-down regulatory pathways in coastal systems where local predator populations have been substantially reduced but not completely extirpated. However, in kelp forests and other subtidal settings, this inherent lack of density dependence can also make trait-mediated trophic cascades difficult to detect based solely on visual count data, leading to limited assessment of TMIIs in the field (Schmitz et al. 2004). Still, recent manipulative studies conducted on intertidal food webs have suggested that trophic cascades previously attributed to consumptive effects (e.g., green crab-snail-algae, Lubchenco 1978) may actually be driven by masked trait-mediated interactions (Trussell et al. 2002), and a meta-analysis of predator-prey mesocosm experiments conducted by Preisser et al. (2005) found the net effects of intimidation (TMII) to be consistently greater than or equal to direct consumption, accounting for up to 85% of total predator-mediated effect strength in three-level food chains. This has led to speculation that TMIIs may be a critical and underestimated component of predator-driven trophic regulation within subtidal rocky reef ecosystems, and one that requires further evaluation (Schmitz et al. 2004).

For rocky reef ecosystems in California, trophic interaction models identify both urchins and mid-size gastropods (primarily subtidal turban snails *Tegula* spp.) as the two benthic herbivore groups most likely to confer disproportionate per-capita grazing impacts on regional *M. pyifera* populations (Sala and Graham 2002). Like urchins, *Tegula* spp. are voracious and potentially destructive macroalgal grazers that are strongly associated with native macrophytes and highly abundant in kelp forest habitats throughout the central California coast (e.g., Steinberg 1985, Chess 1993, Van Alstyne et al. 1999). In addition, three of five native subtidal species (*T. brunnea, T. montereyi, T. pulligo*; hereafter *Tegula*) show potential to alter their algal foraging preferences in response to benthic predation threat (*Pisaster* spp.*, Pycnopodia helianthoides* sea stars), which suggests that top-down regulation of this grazer group may include a strong non-consumptive (TMII) component (Watanabe 1984a, 1984b). In natural rocky reef environments, subtidal *Tegula* are predominantly found utilizing vertical habitat on fronds of *M. pyrifera* for both foraging and refuge (Watanabe 1984a), even though laboratory feeding trials indicate higher somatic growth and gonad development on a mixed diet containing benthic understory algae (Watanabe 1984b). If snails in natural settings are preferentially consuming a nutritionally suboptimal food source to avoid foraging on the substratum when predators are present, regulatory release of *Tegula* grazing activity due to predator removal could drive cascading effects on local macroalgal communities by eliciting not only a short-term behavioral response (i.e., shift in algal species consumption), but also a potential long-term impact on local grazer population dynamics (if restricted benthic foraging opportunities limit reproductive potential). Despite this, existing attempts to characterize trophic cascades in the region have focused almost exclusively on the consumptive (DMII) effects of two commercially harvested urchin predators (California sheephead, spiny lobster; Hamilton and Caselle 2015, Malakhoff and Miller 2021), and potential regulatory pathways controlling *Tegula* grazing impacts have been largely overlooked.

Predatory sea stars account for > 90% of predator-induced mortality in subtidal *Tegula* populations in central California (Watanabe 1984a), and until recently, these echinoderms were ubiquitous and highly abundant throughout regional kelp forests. However, the 2013 outbreak of sea star wasting disease (SSWD) along the northeastern Pacific presented a unique opportunity to conduct a natural experiment quantifying predator-*Tegula* grazer regulation on an ecologically relevant, reef-wide scale not otherwise achievable with conventional experimental studies. Unlike manual predator manipulations that are logistically limited in duration and scope, disease-induced removal of both main *Tegula* predators (*Pisaster* spp*., P. helianthoides*) persisted on many central California reefs for multiple years after the initial SSWD outbreak, and at the time this research was conducted in 2015-2016, the spatial configuration of disease occurrence was regionally widespread but remained locally patchy. This provided ideal conditions for a natural experiment because sites with severe outbreaks (80-100% sea star loss; designated as SS-) were often adjacent to sites maintaining at least moderate baseline sea star densities (SS+), facilitating a direct comparison of snail grazing behavior and population dynamics on reefs that varied substantially in long-term predator exposure but were otherwise similar in habitat and geographic location. Utilizing a paired sampling design, we conducted visual surveys and field collections of *Tegula* from three sets of (SS+/SS-) sites spanning the central California coast, allowing us to evaluate reef-level effects of predatory sea stars on snail foraging preferences (i.e., macroalgal association), dietary composition, and life history characteristics, ultimately providing a rare opportunity to examine both short-term (behavioral) and long-term (life-history) removal impacts of a non-fishery targeted mesopredator on wild grazer populations at an ecologically meaningful reef-wide scale.

## METHODS

### ***Species and Study System***

All three species of *Tegula* commonly found in subtidal kelp forests off central California display strong habitat associations with native macroalgae and forage primarily on the regionally dominant *M. pyrifera*, which, despite offering relatively low nitrogen and caloric content, provides effective vertical refuge from benthic predators (Watanabe 1984a). Throughout the region, *T. brunnea* and *T. pulligo* are also among the most abundant invertebrate grazers observed in subtidal kelp beds, with > 500 individuals commonly observed on a single *M. pyrifera* (personal observation), making them ideal model organisms to evaluate alternative top-down regulatory pathways in coastal kelp forest systems.

Prior to the 2013 SSWD outbreak, sea stars (*P. giganteus, P. ochraceous*, *P. helianthoides*) represented almost the sole source (> 90%) of predator-induced mortality for subtidal *Tegula* populations in the region, and in laboratory settings, *T. brunnea, T. montereyi,* and *T. pulligo* all display consistent flight or avoidance behaviors in response to sea star presence (Watanabe 1984b). Species distribution of subtidal *Tegula* within nearshore reefs is also consistent with the vertical predator escape response widely documented for intertidal and subtidal congeners when sea stars are present (Steinberg 1985). Both *T. pulligo* and *T. montereyi* occur at depths where sea stars are common (7-12 m) and utilize vertical habitat on the fronds of *M. pyrifera* almost exclusively*,* while *T. brunnea* occurs at shallower depths where sea stars are rare (0-6 m) and utilizes both *M. pyrifera* and smaller brown/red algae (e.g., *Dictyoneuropsis reticulata, Rhodymenia* spp.) that are higher in nutritional value but accessible to predators

(Watanabe 1984a). Previous research indicates that these patterns of algal habitat use may be driven by a tradeoff between food quality and predation risk, and if this is the case, characterizing the behavioral grazing response of subtidal *Tegula* populations to disease-induced sea star removal may provide valuable insight into the ecological role of non-consumptive predator-grazer regulation (TMIIs) in maintaining macroalgal assemblages on California rocky reefs.

### ***Sampling Design***

To evaluate the short-term (behavioral, dietary) and long-term (reproductive) response of *Tegula* grazers to the presence of their dominant native predators, we employed the 2013 outbreak of SSWD along the California coast as a natural experimental setup to compare macroalgal utilization and dietary composition of wild *Tegula* populations in the presence and absence of predatory sea stars (SS+/SS-), as well as potential differences in reproductive investment for local snail populations with differing predator exposure histories. For this research, we selected paired (SS+/SS-) sites in three central California study regions: Monterey Bay, Big Sur, and San Luis Obispo (Figure 1). Initial site selection within each research area was based on the presence of suitable, *M. pyrifera*-dominated rocky reef habitat, prevalence of SSWD (PISCO; http://data.piscoweb.org/marine1/seastardisease.html), occurrence of proximate sites with mild (SS+) and severe (SS-) disease impacts, and availability of baseline data on sea star densities in the years leading up to the 2013 disease outbreak (PISCO). Prior to field sampling in 2015, preliminary visual surveys were conducted at a number of potential study sites within each region to characterize benthic algal cover and quantify initial densities of predatory sea stars, allowing us to confirm that a) paired (SS+/SS-) sites were effectively comparable in habitat (i.e., macroalgal availability, wave exposure, reef structure) and b) initial classification of sites as high or low predator presence was consistent with current empirical data.

### ***Field Surveys and Sample Collection***

Once appropriate paired study sites were confirmed, quantitative evaluations of macroalgal cover, sea star density, and *Tegula* density and algal association were conducted in all three study regions from September 2015 - January 2016. Although seasonal variability in *Tegula* distribution has not been documented in California populations (Watanabe 1984a), laboratory studies have indicated that metabolic activity and feeding habits of *Tegula* may be influenced by temperature (Yee and Murray 2004). Thus, paired sites within each study area were surveyed on consecutive dates whenever possible, except in the case of San Luis Obispo, where adverse weather conditions necessitated a 14-d gap between visual surveys at SS+ and SS- locations. Because regional sea surface temperatures throughout 2015 and much of 2016 remained anomalously warm due to a strong El Nino Southern Oscillation (ENSO) event, ambient water temperatures recorded during field surveys displayed minimal variability (< 1 $^{o}$C) across the three study regions, and never differed by more than 0.3 $^{o}$C between paired reefs.

At each of the six study sites, pairs of observers on SCUBA quantified densities of sea stars and *M. pyrifera* on rocky reef habitat between 8-11 m depth using 30 m x 2 m transects (n = 6 per site). All sea stars observed on each transect were enumerated by species, and *M. pyifera* density was quantified with visual counts of both individuals and fronds. Benthic *Tegula* counts were performed within six 0.125-m$^2$ quadrats placed at regular 5-m intervals on each transect (total n = 36 per site), and digital photographs were taken of each quadrat and later analyzed in Image J to quantify benthic red and brown algal cover by species or lowest identifiable taxon. All algae present in a given quadrat were also identified in situ, and these were retained as reference for digital analysis. Vertical *Tegula* counts were conducted by each diver on a haphazardly selected *M. pyifera* individual during the descent/ascent portions of each dive on a given transect to satisfy safety constraints in diving profiles (total n = 12 per site). Prior to analysis, all count data (i.e., sea stars, benthic/vertical *Tegula, M. pyrifera*) were converted to units of density (# individuals / $m^2$) for each transect, while benthic red and brown algal abundance were expressed as percentage cover.

After visual surveys were completed at each site, 50 adult *Tegula* were collected haphazardly, along with 10 samples each of *M. pyrifera,* benthic brown algae (*D. reticulata, Stephanocystis osmundacea*), and benthic red algae (*Rhodymenia* spp.*, Callophyllis* spp.) To account for potential bias in dietary composition due to microhabitat collection location, 25 snails were taken from the substratum and 25 from vertical habitat on *M. pyrifera* fronds, and these were tested initially as different treatment groups.

### ***Sample Processing***

In the laboratory, all snail specimens were measured using calipers to determine basal shell diameter, then manually dissected to separate hard shell growth from somatic and reproductive tissue. The sex and reproductive state (ripeness) of each individual was then determined visually based on gross gonad morphology and coloration; female reproductive tissue for *Tegula* is typically dark green, whereas male reproductive tissue is creamy white (Ortiz-Ordonez et al. 2009). To minimize potential confounding effects of demography across sites, *Tegula* samples were limited to include only adult specimens measuring ≥ 15 mm shell diameter. All individuals within this size range were assumed to be reproductively mature based on visual observations of gonad condition as well as the published size at maturity for a common intertidal congener (Paine 1971).

Soft tissue samples (somatic and gonad) were dried separately at 60 °C for a minimum 48 h. All samples were then weighed to the nearest 0.01 g, and estimates of dry-weight biomass for shell growth, somatic tissue, and gonad tissue were recorded for each individual. Proportional allocation to reproductive tissue was evaluated across study sites using gonadosomatic index (GSI), and this was calculated using the following equation:

$$GSI = \frac{Gonad\ Dry\ Weight}{Total\ Soft\ Tissue\ Dry\ Weight}\ x\ 100$$

To conduct dietary stable isotope analysis, dried tissue samples from snails and algae were first lipid-extracted to minimize potential anomalies in $\delta^{13}$C signatures due to variable fat content, then analyzed for $\delta^{13}$C and $\delta^{15}$N using an isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility. With some recent exceptions (e.g., Hamilton et al. 2014), applications of stable isotopes to characterize food web dynamics in temperate kelp forests have been somewhat limited, due in part to inherent ambiguity between suspended kelp detritus and phytoplankton producer signatures (Miller and Page 2012). However, the simplified experimental system evaluated here is ideally suited for an isotopic approach for several reasons. First, the ecological role of *Tegula* as primary consumers grazing directly on benthic macroalgae allowed us to exclude water column-based production sources (phytoplankton, suspended detritus), which are often highly variable and can lead to uncertainty or bias in mixing model interpretation (Miller and Page 2012). Additionally, all three producer groups potentially utilized by snails in the region (*M. pyrifera,* benthic red algae, benthic brown algae) are known to be clearly separated in their isotopic signatures. *M. pyrifera* is markedly enriched in $\delta^{13}$C (≈ -12 to -16‰; Kaehler et al. 2000) relative to both benthic brown algae (*D. reticulata* and *S. osmundacea* ≈ -21 to -22‰) and red algae (*Rhodymenia* spp., ≈ -30 to-31‰; Page et al. 2008), and $\delta^{15}$N signatures, though locally variable based on ambient nutrient levels, tend to be distinct between large macrophytes (i.e., *M. pyrifera*) and benthic understory/encrusting algae within a given location (Page et al. 2008).

### ***Data Analysis***

To assess potential regional variability in the abundance of *Tegula* predators and quantify empirical differences in predator presence between nominal SS+/SS- paired locations, mean transect densities of predatory sea stars (pooled *Pisaster* spp*., P. helianthoides*) were evaluated across study sites using two-way ANOVA with study region and site predator designation (SS+, SS-) as main effects. To determine whether *Tegula* macroalgal habitat use (i.e., grazing behavior) differed based on predator presence, mean transect densities of *Tegula* spp. were analyzed using a nested ANOVA approach, evaluating effects of snail vertical habitat distribution (benthic algae vs. *M. pyrifera*) and predatory sea star presence (SS+/SS- sites, paired within study region).

Because potential long-term dietary impacts of *Tegula* grazing behavior (i.e., vertical distribution) could not be realistically assessed through direct behavioral observation, time-integrated estimates of algal consumption by grazers at SS+/SS- sites were obtained using results of tissue stable isotope analysis. To evaluate the relative consumption of macroalgal taxa providing vertical refuge (*M. pyrifera*) vs. high nutritional quality (benthic understory algae), $\delta^{13}C$ and $\delta^{15}N$ signatures of producers (*M. pyrifera*, benthic red algae, benthic brown algae) and grazers (*Tegula*) were entered into a dual-isotope, three-source mixing model using the SIAR package in R (Parnell et al. 2010) to estimate proportional contributions to *Tegula* diet for each study site (see Fry and Sherr 1989). Mixing models were run using the standard trophic enrichment factor (TEF) of 1.5‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ typically applied to temperate marine food web research (Post 2002, reviewed in Page et al. 2013), with a trophic position of 1 to reflect the role of *Tegula* as a primary consumer, grazing directly on benthic macroalgae.

Potential long-term life history impacts of predatory sea star presence on *Tegula* dietary signatures ($\delta^{13}C$, $\delta^{15}N$), reproductive investment (GSI), and body size distribution (basal shell diameter) for local grazer populations in all three study regions were evaluated using two-way ANOVA with study region and site predator designation (SS+, SS-) as main effects. This statistical approach was selected in order to isolate the specific effects of predatory sea star regulation on local grazer dynamics from any inherent biogeographic variability in *Tegula* populations across regions, given that latitudinal gradients in both morphology and predator avoidance behavior have been previously documented for intertidal congeners (Frank 1975, Fawcett 1984). Similar two-way ANOVA models were also used to evaluate the effects predatory sea star presence on lower trophic levels; *Tegula* and *M. pyrifera* were analyzed using individual densities (no. per 60 $m^2$), and benthic algal groups (total red, fleshy red, encrusting red, brown) were analyzed using percentage cover, calculated by averaging across quadrats within a given transect.

To evaluate potential ecosystem-level impacts of predatory sea star removal at a reef scale, linear correlations between short-term grazing behavior (proportion of snails on *M. pyrifera*), long-term macroalgal consumption (% red algae in diet), and long-term life history responses of local snail grazer populations (i.e., GSI, *Tegula density*) were evaluated in relation to the macroalgal abundance of both preferred (*Macrocystis*, fleshy red algae) and non-preferred (encrusting red algae, benthic brown algae) producer groups across study sites in all three regions (n = 6). Correlations were also run separately for SS+ and SS- locations, although given the low sample size, these were evaluated only visually as a qualitative tool to assess directional trends based on predator presence. A similar correlation approach was applied to compare the strength and directionality of linear relationships between predator abundance (*Pisaster* spp. density), proportion snails on *M. pyrifera* and *Tegula* density on the local abundance of preferred macroalgae (*M. pyrifera*, benthic red algae).

Prior to statistical analysis, all data were evaluated for normality (Kolmogorov-Smirnov) and equality of variance (Levene's test) and transformed as needed to resolve heteroscedasticity issues. In both site- and transect-scale correlation matrices (and associated scatterplots), abundance estimates for predators (*Pisaster* spp.), grazers (*Tegula*), and *M. pyrifera* represent ln-transformed density values, while benthic coverage estimates for understory macroalgal groups represent  untransformed percentage values from quadrat image analysis.

## RESULTS

### *Local sea star population response to disease outbreak*

Across all three study regions (Figure 1A), mean densities of predatory sea stars were below historical values (Yellin et al. 1977, Watanabe 1984), even at SS+ sites (selected based on mild to moderate SSWD outbreak). Densities at SS+ sites ranged from 3.3 ± 0.8 SE individuals observed per 60 m2 transect in Big Sur (BIS), 4.7 ± 1.7 SE in Monterey (MON), and 11.8 ± 2.6 SE in San Luis Obispo (SLO; Table 1). Predator assemblages at all three SS+ sites were represented entirely by the genus *Pisaster* (primarily the giant-spined star *P. giganteus*), and no *P. helianthodes* were observed either on or off transects at any of the six study sites during field surveys; thus, the density of *Pisaster* spp. is considered to be representative of predator presence for all subsequent analyses.

Despite observed regional variability (driven largely by SLO; $F_{2,27} = 3.80$, $P = 0.04$; Table 2), *Pisaster* densities in all three study regions were consistently and substantially higher at designated SS+ sites compared to paired SS- locations ($F_{1,27} = 96.58$, $P < 0.001$), validating our application of SSWD as a natural experimental setup to evaluate *Tegula* grazer release. SS- sites were chosen specifically to target areas of severe SSWD outbreak in hopes that disease-driven mortality would achieve near to complete local extirpation of predatory sea stars, and within each of the three study regions, only a single predatory *Pisaster* sea star was observed across all SS- transects. In contrast, densities of non-predatory sea stars (pooled across species not known to regularly predate upon Tegula) appeared to be relatively unaffected by local SSWD occurrence. The non-predatory sea stars displayed substantial regional variability (F2,27 = 30.26, P < 0.001, Table 2), minimal difference between paired SS+/- sites (F =, P = 0.054, Table 2), and actually occurred in slightly higher densities at SS- sites in all three study regions (F1,27 = 4.06, P = 0.05; see Table 1).

### *Tegula behavioral grazing response to predator removal*

Despite significant regional variability in observed densities of *Pisaster* spp. across SS+ sites, local *Tegula* populations in all three study regions showed a consistent behavioral response to predator presence in visual surveys: snails increased utilization of benthic understory algae at sites where predation threat was effectively removed (Figure 1B, C). Proportional *Tegula* algal association at all three SS+ sites was similar to published historical field distributions (Watanabe 1984a) with a large majority of individuals (89-92%) observed on vertical structure provided by giant kelp *Macrocystis pyrifera*. In contrast, 48-53% of *Tegula* at SS- locations were observed grazing on benthic red and brown algae. Snails in general appeared to be tightly associated with algal structure, and individuals counted in benthic quadrats were rarely, if ever, observed on bare substrata.

To evaluate potential shifts in algal habitat utilization between SS+/- locations, *Tegula* density on transects was initially analyzed separately between benthic habitat (extrapolated from quadrats) and vertical habitat (extrapolated from *M. pyrifera* counts), revealing significant effects of both predator presence (SS+/-, nested within region) and vertical habitat position. In general, snails were more abundant at sites with predatory sea stars removed (ANOVA, $F_{5,59} = 11.18$, $P < 0.001$), and overall densities were greater on giant kelp compared to benthic vegetation ($F_{1,59} = 30.31$, $P < 0.001$). However, there was also a significant interaction between predator presence and snail vertical distribution ($F_{5,59} = 6.51$, $P < 0.001$), and subsequent post-hoc pairwise comparisons of benthic vs. vertical *Tegula* density within each study site revealed that observed vertical habitat effects (i.e., greater densities of snails on *M. pyrifera*) were only evident at sites with predators present ($P < 0.05$; Figure 1B). Snail densities at all three SS- sites were similar between vertical and benthic algal habitats; indicating that preferential utilization of vertical structure (i.e., *M. pyrifera*) may not maintained when predatory sea stars are removed.

Although *Tegula* in all three study regions displayed a clear behavioral response to near or complete local predator extirpation (SS- sites), regulatory effects of *Pisaster* presence on snail algal utilization at SS+ locations were notably density-independent at both a reef and transect scale. Despite the significant regional variability in SS+ *Pisaster* density noted above, the proportion of snails observed on *M. pyrifera* was consistently higher at SS+ over paired SS- sites (ANOVA, $F_{1,27} = 86.17$, $P < 0.001$), with no significant regional effects ($F_{2,27} = 0.256$, $P = 0.776$) or interactions ($F_{2,27} = 0.737$, $P = 0.488$). Tegula at all three SS+ sites displayed similarly high (≈ 90%) proportional utilization of *M. pyrifera* while *Tegula* at all three SS- sites were distributed fairly evenly (≈ 50%) between *M. pyrifera* and benthic algal habitat (Figure 1C). At a transect level, *Pisaster* density within SS+ sites also showed considerable variability (ranging from a single predatory sea star to > 20 on a 60 $m^2$ transect; Figure 1D), but again, no significant relationship was detected or even qualitatively apparent between local *Pisaster* density and *Tegula* vertical distribution (i.e., proportion on *M. pyrifera*; $r = -0.131$, $p = 0.603$). Instead, local snail populations appeared to display a relatively bimodal grazing response to the overall presence/absence of predators in the general area (i.e., SS+/SS-) rather than scaling their grazing behavior (i.e., utilization of "riskier" benthic algal sources) to specific local predator densities at either a reef or a transect scale. It is notable that even though the lowest transect *Pisaster* densities within SS+ sites (i.e., 1 / 60 $m^2$) resembled observed predator densities (or lack thereof) at SS- sites, vertical habitat association of snails on these transects remained similar to other SS+ replicates (Figure 1D).

Although not sensitive to *Pisaster* density, the observed *Tegula* grazing response to echinoderm presence did appear to be species-specific, with snails distinguishing predatory sea stars (i.e., *Pisaster*) from other locally abundant species that do not typically pose a predation threat. Within our study area, several common "non-predatory" taxa (primarily *Patiria* with occasional *Leptasterias,* and *Dermasterias*) remained highly abundant throughout both SS+ and SS- study locations, with densities nearly an order of magnitude higher than local *Pisaster* populations. However, no significant relationship between non-predatory sea star density and snail vertical distribution was detected for transects at either SS+ or SS- sites ($r = 0.090$, $p = 0.726$; $r = 0.322$. $p = 0.277$), and increased utilization of benthic algae by *Tegula* at SS- locations did not appear to be affected by the presence of these non-predatory taxa, despite local densities in some cases exceeding 50 individuals on a single 60 $m^2$ transect (Figure 1E).

### *Long-term effects of behavioral grazing shifts on Tegula diet*

Results of stable isotope analysis (SIA) suggest that observed behavioral differences in snail vertical distribution between paired SS+/SS- sites in short-term visual surveys are also directly reflected in the long-term proportional dietary contributions of *M. pyrifera* vs. benthic algal sources supporting resident *Tegula* populations within a given site.

All three primary producer groups potentially contributing to *Tegula* diet in the current study were well separated in tissue $\delta^{13}C$ signatures (Table 3), with values for *M. pyrifera* (≈ -14-15‰) substantially enriched compared to both benthic red (≈ -30-32‰) and benthic brown (≈ -21-22‰) algal sources across all three study regions. Tissue $\delta^{15}N$ signatures were relatively similar between *M. pyrifera* and benthic red algae (≈ 7.5-8‰), but were more depleted for benthic brown algae (≈ 6‰), which provided discrimination in mixing models, given that $\delta^{13}C$ signatures for benthic brown algae fell almost directly intermediate between the other two producers (see Figure 2A). Producer $\delta^{13}C$ and $\delta^{15}N$ signatures for *M. pyrifera* did not differ significantly across the three study regions ($F_{2,54} = 1.91, 1.50, P = 0.16, 0.23$). However, benthic red and brown algae displayed significant regional variability in both $\delta^{13}C$ ($F_{2,54} = 26.84, 4.94, P < 0.001; P = 0.011$) and $\delta^{15}N$ ($F_{2,54} = 20.95, 5.20\ P < 0.001; P = 0.009$); consequently, SIAR mixing models for paired SS+/SS- sites were eventually run using separate sets of producer signatures for each study region, based on the values presented in Table 3.

*Tegula* isotopic signatures displayed substantial individual variability (Figure 2A-B) but also revealed a distinct shift between SS+ and SS- sites. Once corrected for isotopic fractionation (using $\Delta^{13}C = 1.5‰$, $\Delta^{15}N = 3.4‰$; estimated trophic position [TP] = 1), individual *Tegula* $\delta^{13}C$ values generally fell between *M. pyrifera* and benthic algae signatures (Figure 2A). However, snails collected from SS- sites were substantially more depleted in $\delta^{13}C$ (indicating stronger benthic contribution) and also displayed higher variability (scatter) compared to SS+ samples, which displayed tighter clustering and relatively enriched $\delta^{13}C$ signatures that more closely resembled *M. pyrifera*). Increased variability of *Tegula* dietary signatures in the absence of predators is also clearly evident in the standard deviations of raw (uncorrected) *Tegula* spp. $\delta^{13}C$ values (Table 3), which ranged from just ± 0.7-1.4‰ at SS+ sites, but ± 3.1-6.4‰ at SS- locations. Overall, mean grazer $\delta^{13}C$ signatures displayed a consistent response to predator presence (SS+/-; ANOVA, $F_{1,294} = 100.04$, $P < 0.001$), with snails collected from SS+ locations showing significant enrichment over paired SS- sites in all three study regions (Figure 2B). A significant regional effect was also detected, indicating significant depletion in SLO compared to BIS and MON ($F_{2,294} = 8.835$, $P < 0.001$); however, this was only evident at SS- locations (predator x region; $F_{2,294} = 6.506$, $P = 0.002$).

In contrast, *Tegula* $\delta^{15}N$ signatures failed to show any significant effect of predator presence and varied only by region ($F_{2,294}= 7.74$, $P = 0.001$), displaying slightly depleted values in BIS compared to MON and SLO, but no discernable difference between paired SS+/- study sites (Figure 2C). Still, inclusion of $\delta^{15}N$ was critical for improving source discrimination between benthic producers in *Tegula* diet, given that C signatures for both benthic (red and brown) algal groups were depleted relative to snail values. Here, results from all six study sites suggest that *Tegula* consumption of benthic brown algae may be relatively minimal; corrected $\delta^{15}N$ signatures for a majority of SS+ and SS- snail samples were quantitatively similar to both *M. pyrifera* and benthic red algae (Figure 2A), but remained consistently enriched (in most cases, by at least 0.5 – 1.0‰) compared to benthic brown algae, even after subtracting the standard trophic correction factor (TEF, $\Delta^{15}N = 3.4‰$) ($\Delta^{15}N = 3.4‰$; Post 2002, reviewed in Page et al. 2013, see additional validation in Appendix 1).

This was reflected in SIAR mixing model results for all three study regions (Figure 2D), which found *Tegula* diet at paired SS/- sites to display consistent proportional shifts in the relative contributions of benthic red algae and *M. pyrifera*, with no substantial benthic brown algae contribution. Grazer diet at all three SS+ sites was dominated by *M. pyrifera*, with contribution estimates ranging from 0.80 at SLO to 0.70-0.72 at MON and BIS. Benthic red algae was ranked second in dietary importance, although estimated contributions at SS+ locations were relatively minimal (0.17-0.19), followed by benthic brown algae at 0.10-0.13 (MON, BIS) and 0.03 (SLO). In contrast, *Tegula* from SS- locations were estimated to receive substantially higher dietary benthic input. At each of the three SS- sites, *M. pyrifera* was still identified as the most heavily consumed food source, but estimated dietary contributions from *M. pyrifera* (0.49-0.55) were consistently lower than at paired SS+ locations, and snails in the absence of predators also received substantial production from benthic red algae, which represented well over a third of estimated dietary contribution at MON (0.39) and BIS (0.37) and nearly half of total dietary contribution at SLO (0.47). Estimated SS- contributions from benthic brown algae remained low (0.04-0.09) at SS- sites in all three study regions.

### *Potential long-term effects on Tegula life history characteristics and local population dynamics*

Gross dissection of individual *Tegula* (total n = 300) collected from each region revealed significant differences in grazer reproductive allocation (i.e., gonadosomatic index (GSI)) between paired SS+/- sites. Male: female sex ratio did not differ significantly from 50:50 in individual snail samples (n = 50) taken from any of the six study sites (Chi-square test, $p > 0.05$); however, *Tegula* from SS- sites displayed a larger percentage of individuals with ripe gonads (> 80%), compared to SS+ samples (≈ 60-66%). Mean GSI was higher at all three SS- sites (0.24-

0.25 [MON, BIS], 0.21 [SLO]) compared to paired SS+ locations (0.17 – 0.18; Figure 3A). Even after accounting for proportional increases in gonad allocation with snail body size (using shell diameter as a covariate; ANCOVA, $F_{1,293} = 32.97$, $p < 0.001$), there was a significant negative effect of predatory sea star presence on snail GSI ($F_{1,293} = 52.32$, $p < 0.001$) that was quantitatively consistent across all paired SS+/- sites, with no significant regional effect ($F_{2,293} = 2.062$, $p = 0.109$) or region x predator interaction ($F_{2,293} = 1.161$, $p = 0.315$). The mean GSI of *Tegula* across both SS+ and SS- sites displayed a strong positive correlation with % dietary contribution of benthic red algae in snail diet, as estimated by SIAR mixing models ($r = 0.984$, $n = 6$, $p < 0.001$; Figure 3B).

Size frequency distributions of basal shell diameter for *Tegula* samples in all three study regions also revealed consistent differences in the size demographics of local *Tegula* populations between paired SS+/- locations (Figure 3C). Mean shell diameter was similar across regions ($F_{2,294} = 3.791$, $p = 0.393$) but displayed a significant response to predator presence ($F_{1,294} = 62.14$, $p < 0.001$), and both mean and median size values were consistently larger for snail samples at sites with predatory sea stars removed by SSWD (SS-; mean: 24.7-25.2 mm, median: 24-25 mm) compared to sites where some predators remained present (SS+; mean: 22.2-23.0, median: 22-23 mm). *Tegula* body size distributions from SS- sites exhibited a larger proportion of individuals in smaller size classes compared to SS+ samples, particularly evident within the 16-20 mm size range roughly corresponding to age class -1 and -2 individuals (see Paine 1971), which would ostensibly represent local snail recruitment in the time frame following disease-induced predator removal at SS- sites, given that 2015 field collections were conducted approximately two years after initial SSWD outbreak. In *Tegula* samples taken from SS- sites, 20-26% of individual snails fell within this 16-20 mm size range, suggesting that local grazer populations may be largely composed of younger, newly recruited individuals. In contrast, smaller size class (16-20 mm) individuals represented only 8-13% of snails collected from SS+ sites in SLO and MON, and no snails < 21 mm basal shell diameter were collected from the SS+ site in BIS, suggesting either lower *Tegula* recruitment with predators present or relatively higher densities of large individuals compared to paired SS- sites.

### ***Potential ecosystem effects of Tegula grazer release at multiple trophic levels***

Although evidence based on visual survey data is inherently correlative, patterns of abundance between paired SS+/- study sites for *Tegula* and macroalgal producers were qualitatively consistent with proposed trait-mediated regulatory pathways mitigating impacts of grazing through predatory sea star presence at both short-term (i.e., behavioral) and long-term (i.e., life history) time scales (Figure 4A). In general, both grazers and algal producers displayed a similar numerical response to predator presence across all three study regions, even though predator densities at SS+ sites displayed significant regional variability ($F_{2,27}$= 3.80, P = 0.035; Table 2, Figure 4B).

Mean transect densities of *Tegula* were significantly greater at SS- sites compared to paired SS+ locations in two out of three study regions ($F_{1,27}$ = 14.35, P = 0.001; Table 4, Figure 4B), although similar grazer densities between paired SS+/- sites in BIS resulted in a significant regional interaction effect ($F_{2,27}$= 5.82, P = 0.008).

All three macroalgal producer groups displayed significant regional effects (Table 4, Figure 4B), with generally greater mean values observed in northern locations. *M. pyrifera* transect density and benthic brown algae % cover were significantly greater in MON compared to the other two study regions ($F_{2,27}$ = 7.00, 12.14, P = 0.004, < 0.001, respectively), and mean % cover of benthic red algae was significantly greater in both MON and BIS compared to SLO

($F_{2,27}$ = 15.26, P < 0.001). However, only the two macroalgal groups identified as important food sources for *Tegula* in isotopic mixing models (*M. pyrifera*, benthic red algae) displayed a significant response to predatory sea star presence, and this was in the opposite direction of grazers, with significantly lower mean *M. pyrifera* density ($F_{1,27}$= 4.45, P = 0.04) and total benthic red algae % cover ($F_{1,27}$= 10.19, P = 0.004) observed at all three SS- sites without a significant regional interaction ($F_{2,27}$= 0.36, 0.91, P = 0.70, 0.42). In contrast, percent cover for benthic brown algae, which was not heavily utilized by snails in mixing models, did not display a significant predator effect ($F_{1,27}$= 3.05, P = 0.092) and was qualitatively more abundant at SS- sites in all three study regions (Figure 4B). Despite the significantly lower benthic red algal coverage observed at SS- sites, total benthic algae % cover (all taxa combined) also differed only by study region ($F_{2,27}$= 26.64, P < 0.001), suggesting that predatory sea star removal may primarily impact benthic algal communities through shifts in species composition.

A slightly more detailed taxonomic evaluation of quadrat images revealed total benthic red algae coverage on transects to consist of both erect, fleshy understory taxa and encrusting crustose coralline algae that are generally less impacted by grazers (Table 1; see Lubchenco and Cubit 1980). Separate analysis of each taxonomic group suggests that observed regional variability in total benthic red algae cover was driven primarily by encrusting coralline taxa, which differed significantly by region ($F_{2,27}$ = 13.85, P < 0.001, Table 4) but displayed no detectable effects of predator presence ($F_{1,27}$ = 0.00, P = 0.96), while the observed response to predatory sea star removal (i.e., between paired SS+/- sites) was driven primarily by fleshy understory algae which displayed a highly significant predator effect ($F_{1,27}$ = 56.59, P < 0.001) but did not differ significantly by region ($F_{2,27}$ = 3.10, P =0.062).

### ***Evidence of a trait-mediated trophic cascade driven by disease outbreak***

In considering responses at a site scale (Figure 5), *Tegula* vertical grazing behavior (individuals observed on *M. pyrifera*) within a given site was negatively correlated with both SIAR contribution estimates of benthic red algae in their diet (r = -0.93, P = 0.007) and local reproductive investment (GSI; r = -0.90, P = 0.014; Figure 5). However, this appeared to be driven largely by a bimodal response to predator presence; within each site type (SS+ or SS-), relationships between *M. pyrifera* utilization and benthic red algae consumption/ GSI were qualitatively positive and generally weak, due in part to the low sample size (n = 3 sites) within each predator designation. The direct relationship between benthic red algae consumption and snail GSI was much more consistent, displaying a significant positive relationship overall (r = 0.98, P < 0.001), but also similar positive qualitative relationships within both SS+ and SS- sites, Densities of *Tegula* displayed a qualitative negative relationship with vertical *M. pyrifera* habitat use ( r = -0.41, P = 0.423) and somewhat stronger, albeit non-significant, qualitative positive relationships with both benthic red algae consumption (r = 0.62, P = 0.185) and local GSI (r = 0.72, P = 0.108). Although the inverse relationship between snail grazing behavior (vertical utilization) and *Tegula* density appeared to be a bimodal response to predator presence , the direct relationship between *Tegula* density and GSI was reflected by similar positive linear responses within both SS+ and SS- sites.

At a site scale, both macroalgal producer groups (*M. pyrifera*, benthic red algae) displayed positive relationships with *Tegula* vertical habitat use of *M. pyrifera* and negative relationships with estimated benthic red algae consumption (Figure 5). However, results were only significant for fleshy red algae, which displayed a stronger linear response to both vertical grazing behavior (r = 0.92, P = 0.009) and dietary red algae contribution (r = -0.87, P = 0.025) compared to *M. pyrifera* (r = 0.46, P = 0.356; r = -0.37, P = 0.473). Again, linear responses of both preferred algal groups to behavioral grazing shifts (vertical habitat use) appeared largely bimodal, driven primarily by categorical differences between SS+/- sites. However, particularly for fleshy red algae, the negative relationship between dietary red algal consumption and local algal abundance was also observed within SS- but not SS+ sites. Preferred macroalgal food sources displayed opposite, albeit non-significant responses to local *Tegula* density. *M. pyrifera* displayed a positive correlation overall with local *Tegula* density (r = 0.41, P = 0.423) that was also evident within both SS+ and SS- sites. In contrast, fleshy benthic red algae displayed a weak negative correlation (r = - 0.27, P = 0.612 ) that appeared to differ based on predator presence, with a qualitative positive relationship observed within SS+ locations and a nearly flat linear relationship within SS- sites, suggesting low local abundance in the absence of predators regardless of grazer density. In contrast, neither of the non-preferred macroalgal producer groups (coralline red algae, benthic brown algae) displayed significant or even qualitatively trending relationships with either vertical grazing behavior (proportional usage of *M. pyrifera*) or benthic red algae in grazer diet across sites. However, benthic brown algae was the only macroalgal producer to show a significant linear response to *Tegula* density, displaying a strong positive relationship (r = 0.83, P = 0.04) that was qualitatively consistent within both SS+ and SS- sites.

## DISCUSSION

Much of the debate over the role of top-down regulation in coastal marine systems stems from observed variability in the strength of predator-grazer-producer densities in field research. Although strong correlative results in some studies suggest substantial predator-mediated grazer suppression (e.g., Tuya et al. 2004), research conducted in similar systems often fails to detect clear associations between predator density and grazer/algal abundance, leading to the conclusion that primary producers in these systems are regulated by mechanisms other than predation (e.g., wave disturbance, nutrient runoff; Shears et al. 2008). Still, in certain coastal food webs where fishery activity has led to long-term regional declines in predator abundance (e.g., otter-urchin-*Alaria* kelp forests; Estes et al. 1998, finfish-crab-*Spartina* salt marshes*,* Altieri et al. 2012), widespread, ecosystem-scale losses of vegetative biomass have been attributed to increased grazing impacts, providing compelling evidence that disruption of top-down, predator-mediated regulation can induce potentially catastrophic cascading effects on foundation species. (e.g., kelp) comprising temperate subtidal habitats. This highlights a critical gap in our knowledge of top-down control on temperate reefs. It is likely that an incomplete mechanistic understanding of predator-mediated interactions in subtidal systems is at least partially responsible for the variable detection of top-down control reported across studies, and this may be due to systematic underestimation of non-consumptive, trait-mediated predatory effects (i.e., TMIIs) that are not clearly reflected by numerical relationships in visual field surveys (Schmitz et al. 2004, Byrnes et al. 2006). Because of logistic constraints on conducting experimental predator manipulations in open marine environments, mechanistic field examinations of non-consumptive (i.e., trait-mediated) top-down regulation within subtidal systems have been relatively rare. Opportunities to study long-term or large-scale, ecosystem impacts of predator removal at a population-level, or reef-wide scale have been even rarer, particularly for predatory species that cannot be accurately evaluated across a known gradient in fishing pressure (Frank et al. 20007, Baum and Worm et al. 2009)

Our results here are notable in that the extended multi-year duration of disease-driven sea star extinctions allowed us to utilize a multidisciplinary approach quantifying not only short-term (behavioral) shifts in *Tegula* grazing activity due to predator removal, but also potential long-term population-level impacts on both *Tegula* and algal producers. There was a consistent behavioral grazing response of *Tegula* to local predatory sea star extirpations, with ≈ 90% of snails at SS+ sites prioritizing vertical refuge on *M. pyrifera* (see Watanabe 1984), but approximately half of all snails at SS- sites using benthic understory algae as a nutritionally superior but relatively risky food source. Shifts in grazing behavior reflected the long-term dietary compositions of *Tegula* samples and SS- snails displayed consistently higher gonad investment compared to individuals from paired SS+ sites, indicating that greater access to high quality food following predator removal at longer time scales could potentially alter local *Tegula* population dynamics by increasing reproductive potential. If so, the results presented here could provide an example of non-consumptive, behaviorally-mediated grazer regulation that is initially driven as a short-term TMII that could eventually result in density-mediated algal grazing impacts due to reproductive consequences at a population level if local predatory sea star extinctions were to persist. This could have complex implications for macroalgal abundance and habitat stability over multiple temporal scales, particularly given that the observed behavioral response of *Tegula* to predatory sea star removal involves a shift in diet from a high biomass, rapidly growing macroalga (*M. pyrifera*) to a relatively low biomass, slower growing food source (benthic red understory algae).

Identifying non-consumptive regulatory pathways can be challenging in marine observational research because, unlike DMIIs, behavioral grazing responses to perceived predation threat (and subsequent effects on algal abundance) are often largely uncorrelated with local numerical predator densities. This is particularly true for benthic herbivores such as *Tegula* spp. in kelp forest systems that lack the visual acuity and/or physical mobility to accurately assess local predator numbers in surrounding habitat and scale their threat response accordingly (Bracis and Wirsing 2021). Instead, they may rely on the presence/absence of chemical cues diluted in the water column to initiate a behavioral avoidance response, recently demonstrated for the congener *T. funebralis* in the rocky intertidal (Murie and Bourdeau 2019). Concordantly, subtidal *Tegula* populations in all three regions here displayed consistent and quantitatively similar shifts in proportional *M. pyrifera* use between paired SS+/SS- sites, even though local predatory sea star densities at SS+ locations differed substantially by region. Additionally, despite the complete absence of *P. helianthoides* in field surveys as well as local *Pisaster* densities at two of three SS+ sites that fell well below the historic range (reported as up to 18 individuals per 100 $m^2$ for *P. giganteus*; Watanabe 1984), observed algal habitat use by *Tegula* at SS+ sites was similar to published field distributions for *T. brunnea, T. pulligo, and T. montereyi* off the Monterey peninsula decades prior to any wasting disease outbreak (Watanabe 1984). These results suggest that *Pisaster* may impose behaviorally-mediated regulation of *Tegula* grazing activity that is largely independent of predator density and may be maintained by relatively low levels of physical predator presence, which has positive implications for regional kelp forest resilience to even severe wasting disease outbreaks, as long as predatory sea stars do not experience complete local extirpation.

In addition, the complete lack of a *Tegula* behavioral response to non-predatory sea star species (relatively abundant at both SS+ and SS- sites) provides further support that observed grazing shifts between local SS+/- snail populations represent an adaptive predator avoidance behavior rather than a generalized tactile or sensory response to overall echinoderm presence. This is consistent with species-specific predator recognition of *Pisaster* and *P. helianthoides* by *Tegula* species evaluated in the current study (Watanabe 1983) and for herbivorous gastropods in other systems (see Schram et al. 2019). Along the California coast, vertical predator avoidance responses have been well documented for intertidal *Tegula* congeners (primarily *T. funebralis*) at multiple spatial scales, with snails seeking vertical refuge in erect macroalgae (e.g., *Pterygophera californica*) and using emersed habitat above the waterline to avoid *Pisaster* and *Pycnopodia* sea stars in enclosed tide pools (McClure 2019). Distinct latitudinal gradients in vertical intertidal zonation and size distribution have also been documented in response to regional variability in predation threats (Fawcett 1984), including in response to sea star wasting disease (Gravern and Morgan 2017). *Tegula* species native to kelp forests in both southern (*T. aureotincta*; Schmitt 1987) and northern California  display flight behaviors in response to tactile stimulation by predatory sea stars. Localized reef-scale predatory sea star extinctions maintained by SSWD provided the first opportunity to evaluate both long-term and population-level impacts (Chapter 3) of predatory sea star regulation on local *Tegula* grazers and their macroalgal food sources.

In natural systems with predators present, consumptive and behavioral grazer regulation typically operate simultaneously, which can make it difficult to distinguish between effects of DMII and TMII on algal production and may lead to underestimation of non-consumptive regulatory pathways masked by density-driven effects (Matassa and Trussell 2011, Trussell et al. 2017). Accurate assessment of potential trait-mediated trophic cascades in marine systems requires observed behavioral grazer responses to predator presence to be quantitatively related to specific algal consumption rates over time (see Gravern and Morgan 2019), which can be difficult to establish given that physical algal habitat utilization is often not directly proportional to diet (e.g., Goecker and Kåll 2003). This is particularly true for marine gastropods, which have been shown in a number of systems to preferentially forage and actively avoid certain algal taxa based on physical and chemical herbivory deterrents in marine algae (Pereira et al. 2015), which may have been a factor driving the minimal contribution of benthic brown algae to *Tegula* diets at all six study sites here. However, in general, tissue $\delta^{13}C$ results demonstrated a clear relationship between the observed behavioral response to disease-driven predatory sea star removal  (i.e., decreased use of vertical refuge on *M. pyrifera* at SS- sites) and local dietary composition, with SS+ snails in all three study regions receiving an estimated 70-80% dietary contribution from *M. pyrifera* compared to 39-47% from benthic red algae at paired sites with predatory sea stars removed (SS-). This is somewhat notable given that all three species of *Tegula* evaluated here (in studies conducted prior to wasting disease outbreak) have shown a consistent dietary preference for *M. pyrifera* in laboratory feeding assays (Watanabe 1984), suggesting that snails in natural settings display some degree of adaptive plasticity in dietary preference, opportunistically using nutritionally superior benthic understory algae when the threat of benthic predation is removed for extended time periods. Stable isotopes provide a valuable tool to reconstruct time-integrated dietary in marine subtidal organisms for which direct observation is logistically unfeasible, and tissue turnover rates for aquatic snails have been shown to range from 82-107 d ($\delta^{13}C$) and 29-176 d ($\delta^{15}N$) for nitrogen (Li et al. 2018)  My results suggest that in the weeks or months prior to collection, *Tegula* at both SS+ and SS- study sites in all three regions foraged more or less proportionally on the algae they were observed to inhabit indicating strong potential for behaviorally-driven shifts to exert cascading regulatory impacts and further supporting the idea that strong association with *M. pyrifera* is driven by perceived threat of benthic predation rather than dietary preference based solely on palatability or adaptive nutritional benefit (see Palmer et al. 2021).

Grazer selection of suboptimal food sources based on the tradeoff between food quality and perceived predation risk is a well-established paradigm in optimal foraging theory (Nonacs and Dill 1993), However, because adopting nutritionally suboptimal foraging strategies to minimize predation risk comes at an energetic cost, this can have substantial long-term impacts on local population dynamics, particularly if restricted access to high quality food sources acts as a limiting factor regulating growth and reproduction (see Hebblewhite and Merrill 2009, 2011). Results of reproductive analysis in the current study are qualitatively consistent with long-term laboratory feeding experiments conducted by Watanabe 1984a, which found all three species of *Tegula* evaluated here achieved optimal somatic growth and gonad development when fed on a mixed diet that included benthic understory algae despite their strong preference for a single alga diet of only *M. pyrifera*. Despite its rapid vertical growth and value as structural refuge, *M. pyrifera* is lower in nutritional value and caloric density has a greater ash content compared to the majority of other algae in the study region (, see Watanabe 1984). In contrast, benthic understory red algae (Phylum Rhodophyta) in central California kelp forests consistently display the highest nitrogen content across a majority of algal taxa (Neighbors and Horn 1981) but offer limited protection from predatory sea stars; tethering studies conducted in subtidal kelp forests in Monterey Bay revealed only a 15-30% reduction in *Tegula* mortality provided by benthic understory algae cover compared to bare substrate (Watanabe 1984).

Behavioral regulation of *Tegula* foraging activity based on avoidance of benthic predators (primarily *Pisaster* and *Pycnopodia* sea stars) has previously been suggested as a possible explanation for the apparent mismatch between theoretically optimal snail diet and empirical algal utilization displayed by subtidal *Tegula* in both field surveys and laboratory feeding experiments (i.e., strong habitat association and dietary preference for *Macrocystis* Watanabe 1984a, b). However, unlike earlier research conducted in the decades prior to the outbreak of wasting disease when *Pisaster* were ubiquitous and *Tegula* foraging preferences for *Macrocystis* were uniformly shaped by some degree of predator exposure, SS- sites in the current study provide wild populations of *Tegula* nearly two years of continuous predator absence. At these locations, clear shifts in both algal preference and reproductive investment provide strong evidence that increased access to benthic food sources increases local grazer reproductive investment, and, therefore, the reproduction of *Tegula* in nature may be limited by consuming suboptimal diets of *Macrocystis* in response to the threat of benthic predation. As a result, short-term behavioral regulation of *Tegula* grazing activity may also have top down regulatory effects on grazer population densities at longer time scales, which may have important positive implications for ecosystem stability because the presence of predatory sea stars could induce density-dependent regulation of lower trophic levels even at consumption  rates too low to reduce the densities of grazer populations. However, on the flip side, if predators are completely removed for extended time periods, it could exacerbate cascading effects on lower trophic levels by driving a trophic cascade involving both immediate, short term (behavioral) and longer term (grazer density) effects at different time scales.

Although combined results of my visual surveys (i.e., behavioral observations), dietary stable isotope analysis, and gonadal investment indices (i.e., GSI) indicate a clear relationship between predatory sea star removal, access to high quality benthic food sources, and increased reproductive potential for local snail populations, long term consequences of predator removal for local grazer population dynamics and abundance are less clear. Grazer densities were significantly greater at SS- sites compared to paired SS+ sites in two of three study regions, and snail body size distributions (based on basal shell diameter) indicate that this may be largely driven by increased presence of younger individuals (estimated age class 2 and below), directly corresponding to recruitment years after the initial SSWD outbreak. However, recruitment success can be influenced by several ecological processes (i.e., increased reproduction or larval delivery, increased survival, decreased predation) and the specific dynamics driving increased recruitment success at SS- sites are not unambiguous here. Regardless of the cause(s) and/or scale(s) at which reproduction and grazer densities are linked, observed shifts in grazer populations between paired sites suggest that more widespread predator removal (e.g., complete disease outbreak at a regional scale) could potentially have density-dependent effects on lower trophic levels if disease-driven extirpation of *Pisaster* and *Pycnopodia* persists over longer time periods.

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# TABLES

**Table 1.** Relative transect abundance (mean ± SD) of predatory and non-predatory sea star groups, grazers (*Tegula* spp.) and macroalgae at paired predator present/absent (SS+/SS-) sites in each study region. Abundance values for sea stars, *Tegula* spp., and *M. pyrifera* represent mean transect densities (number of individuals per 60 m$^2$), and % cover for benthic understory algae was calculated for each transect by averaging digital image analysis results from n = 6 photographic quadrats taken at regular intervals along the transect line.

| | *Monterey (MON)* | | *Big Sur (BIS)* | | *San Luis Obispo (SLO)* | |
|---|---|---|---|---|---|---|
| Sea star density | SS+ | SS- | SS+ | SS- | SS+ | SS- |
| *Pisaster* spp. | 4.7 (1.7) | 0.3 (0.3) | 3.3 (0.8) | 0.2 (0.2) | 11.8 (2.6) | 0.2 (0.2) |
| *Pycnopodia* spp. | -- | -- | -- | -- | -- | -- |
| Non-predatory | 43.5 (14.0) | 63.3 (16.1) | 4.3 (1.4) | 7.2 (0.7) | 15.3 (2.3) | 17.5 (3.1) |
| Grazer density | SS+ | SS- | SS+ | SS- | SS+ | SS- |
| *Tegula* spp. | 1759.5 (429.8) | 2436.1 (212.7) | 707.5 (122.9) | 733.8 (139.0) | 424.3 (129.1) | 1565.8 (178.0) |
| Macroalgal density | SS+ | SS- | SS+ | SS- | SS+ | SS- |
| *M. pyrifera* | 18.0 (4.3) | 11.5 (3.1) | 8.5 (0.9) | 7.2 (0.6) | 8.5 (1.1) | 6.5 (1.0) |
| Macroalgal % Cover | SS+ | SS- | SS+ | SS- | SS+ | SS- |
| Benthic brown | 23.6 (0.8) | 29.2 (4.9) | 18.5 (2.9) | 18.7 (2.2) | 16.9 (1.2) | 19.0 (1.6) |
| Benthic red | 34.8 (1.7) | 24.8 (3.9) | 33.6 (2.5) | 28.5 (2.8) | 21.1 (1.9) | 17.4 (2.1) |
| Fleshy | 12.9 (0.8) | 4.3 (0.5) | 8.9 (0.7) | 4.3 (1.3) | 8.9 (1.5) | 3.5 (0.7) |
| Coralline | 21.9 (1.7) | 20.5 (4.2) | 24.7 (3.0) | 24.2 (1.9) | 12.2 (1.5) | 13.9 (1.6) |
| Benthic (total) | 58.4 (2.0) | 54.0 (4.0) | 51.1 (1.4) | 47.2 (4.6) | 38.0 (2.2) | 36.4 (1.5) |

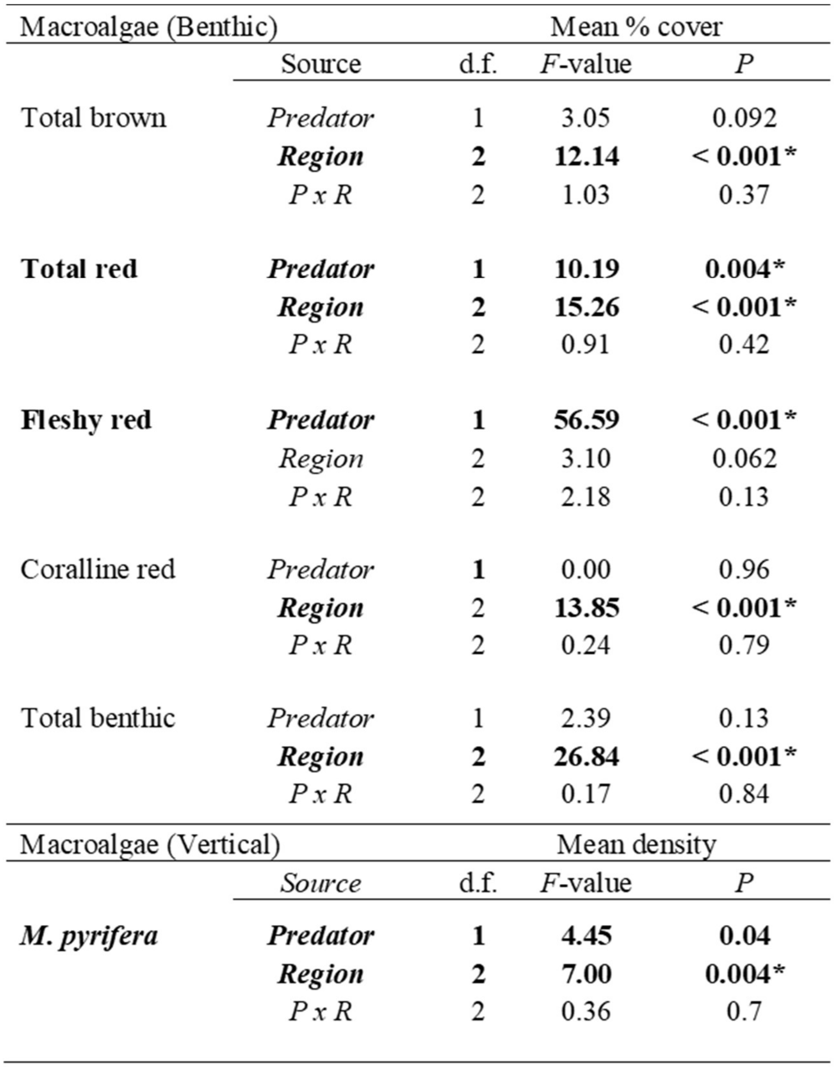
**Table 2.** Results of two-way ANOVA analysis for sea star and grazer (*Tegula* spp.) densities, evaluating main effects of region (MON, BIS, SLO) and predator presence (based on site designation: SS+/SS-). Significant effects for all models are bolded and designated with an asterisk.

| | | | Mean density | |
|---|---|---|---|---|
| | Source | d.f. | *F*-value | *P* |
| *Pisaster* spp. | ***Predator*** | **1** | **96.58** | **< 0.001*** |
| | ***Region*** | 2 | **3.80** | **0.035** |
| | ***P x R*** | 2 | **4.35** | **0.023** |
| Non-predatory sea stars | ***Predator*** | **1** | **4.06** | **0.054** |
| | ***Region*** | **2** | **30.26** | **< 0.001*** |
| | *P x R* | 2 | 0.70 | 0.506 |
| *Tegula* spp. | ***Predator*** | **1** | **14.35** | **0.001*** |
| | ***Region*** | **2** | **12.51** | **< 0.001*** |
| | ***P x R*** | **2** | **5.82** | **0.008*** |

**Table 3.** Isotopic signatures (mean ± SD) of producers (algae) and grazers (*Tegula* spp.) for each of the three study regions. Producer signatures did not differ significantly between paired sites, so values were pooled by study region. Grazer signatures are reported separately for predator present/absent (SS+/SS-) locations within each region.

| Region | Trophic group | $\delta^{13}C$ | $\delta^{15}N$ |
|---|---|---|---|
| Monterey (MON) | Benthic brown algae | -21.0 ± 1.1 | 6.1 ± 0.6 |
| | Benthic red algae | -30.2 ± 1.0 | 8.1 ± 0.4 |
| | *M. pyrifera* | -14.1 ± 0.7 | 7.5 ± 0.7 |
| | *Tegula* spp. *(SS+)* | -16.1 ± 0.7 | 11.1 ± 0.8 |
| | *(SS-)* | -19.2 ± 4.2 | 11.2 ± 1.1 |
| Big Sur (BIS) | Benthic brown algae | -22.1 ± 1.6 | 5.8 ± 0.3 |
| | Benthic red algae | -30.0 ± 0.8 | 7.6 ± 0.6 |
| | *M. pyrifera* | -14.5 ± 1.4 | 7.7 ± 0.3 |
| | *Tegula* spp. *(SS+)* | -16.6 ± 1.4 | 10.9 ± 0.2 |
| | *(SS-)* | -19.2 ± 3.1 | 10.9 ± 0.6 |
| San Luis Obispo (SLO) | Benthic brown algae | -20.8 ± 1.4 | 6.3 ± 0.4 |
| | Benthic red algae | -32.6 ± 1.8 | 7.2 ± 0.3 |
| | *M. pyrifera* | -15.0 ± 1.1 | 7.5 ± 0.3 |
| | *Tegula* spp. *(SS+)* | -16.6 ± 1.3 | 11.2 ± 0.7 |
| | *(SS-)* | -22.3 ± 6.4 | 11.2 ± 0.9 |

**Table 4.** Results of two-way ANOVA analysis of abundance (either density or percent cover) for all macroalgal producer groups, evaluating main effects of region (MON, BIS, SLO) and predator presence (SS+/SS-). Significant effects are highlighted in bold and designated with an asterisk, and macroalgal groups displaying a significant response to predator presence are also bolded.



| Macroalgae (Benthic) | | Mean % cover | | |
|---|---|---|---|---|
| | Source | d.f. | *F*-value | *P* |
| Total brown | *Predator* | 1 | 3.05 | 0.092 |
| | ***Region*** | **2** | **12.14** | **< 0.001*** |
| | *P x R* | 2 | 1.03 | 0.37 |
| **Total red** | ***Predator*** | **1** | **10.19** | **0.004*** |
| | ***Region*** | **2** | **15.26** | **< 0.001*** |
| | *P x R* | 2 | 0.91 | 0.42 |
| **Fleshy red** | ***Predator*** | **1** | **56.59** | **< 0.001*** |
| | *Region* | 2 | 3.10 | 0.062 |
| | *P x R* | 2 | 2.18 | 0.13 |
| Coralline red | *Predator* | **1** | 0.00 | 0.96 |
| | ***Region*** | 2 | **13.85** | **< 0.001*** |
| | *P x R* | 2 | 0.24 | 0.79 |
| Total benthic | *Predator* | 1 | 2.39 | 0.13 |
| | ***Region*** | **2** | **26.84** | **< 0.001*** |
| | *P x R* | 2 | 0.17 | 0.84 |
| **Macroalgae (Vertical)** | | **Mean density** | | |
| | *Source* | d.f. | *F*-value | *P* |
| ***M. pyrifera*** | ***Predator*** | **1** | **4.45** | **0.04** |
| | ***Region*** | **2** | **7.00** | **0.004*** |
| | *P x R* | 2 | 0.36 | 0.7 |

# FIGURES

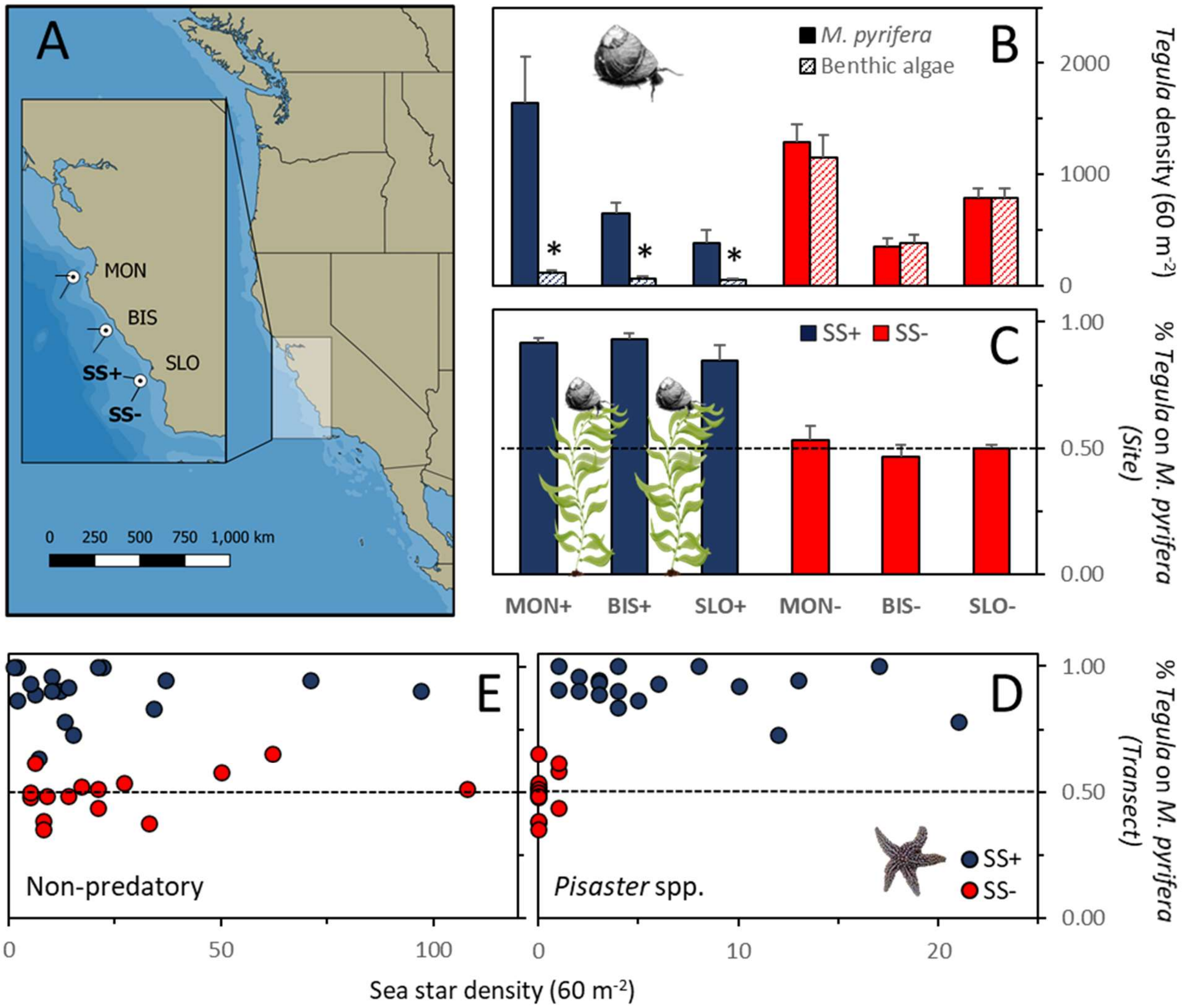


**Figure 1.** (A) Map of paired predator present (SS+) / absent (SS-) study sites in each of the three central California study regions: Monterey (MON), Big Sur (BIS), and San Luis Obispo (SLO), along with the (B) vertical habitat distribution of *Tegula* grazers observed at each study site during field surveys (mean density of snails on *M. pyrifera* vs. benthic understory algae) and (C) the mean proportions of total snails found on *M. pyrifera* at each study site. Scatterplots depict transect-scale relationships between *Tegula* habitat association with *M. pyrifera* (i.e., vertical refuge) and local densities of (D) predatory (*Pisaster* spp.) and (E) non-predatory sea stars. Data points represent the proportions of total snails counted on *M. pyrifera* for individual transects, taken across all SS+ (blue) and SS- (red) locations.

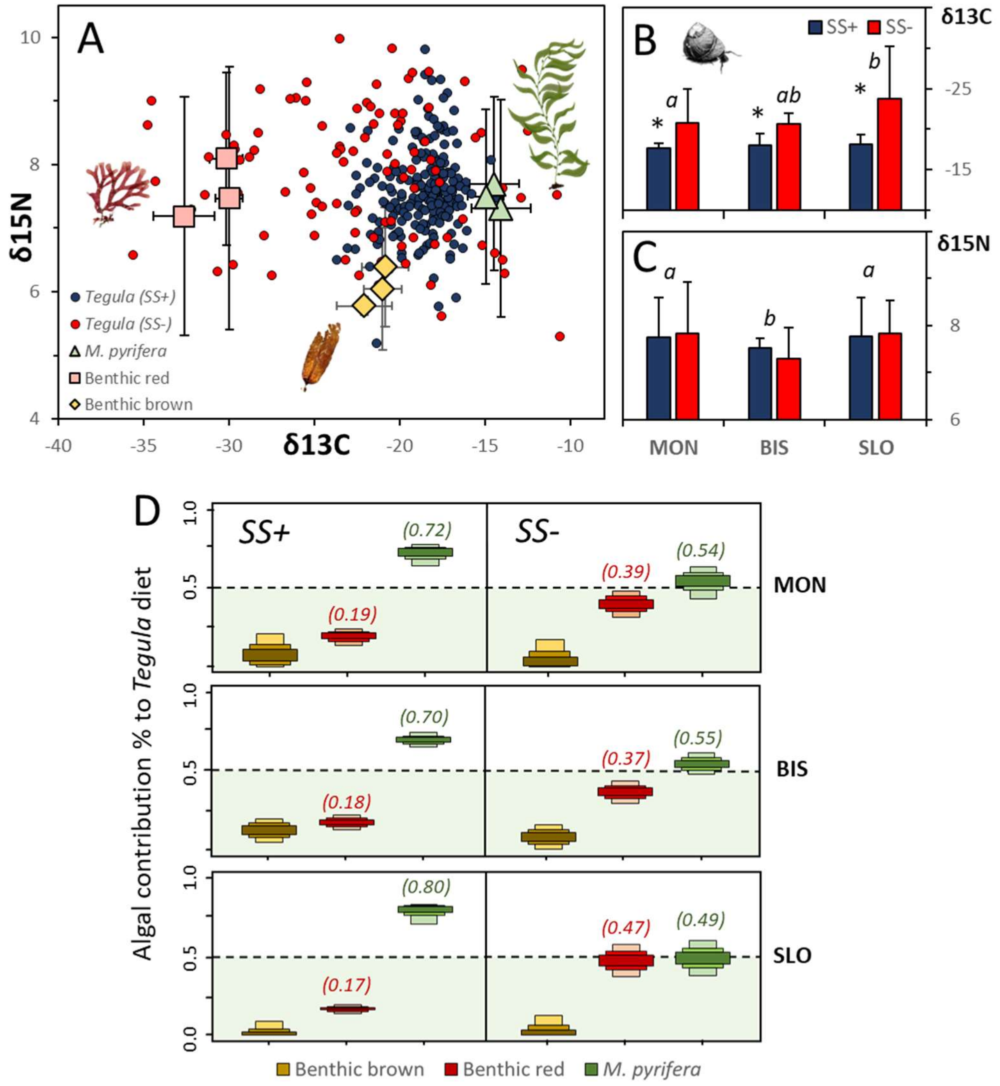


**Figure 2.** (A) Biplot of mean $\delta^{13}$C and $\delta^{15}$N signatures (± SD) for macroalgal producers (*M. pyrifera*, benthic red algae, benthic brown algae) in each study region, with individual grazer signatures (*Tegula* spp.) grouped by predator presence. Bar graphs (B-C) denote mean grazer $\delta^{13}$C and $\delta^{15}$N values by region and study site (SS+/SS-). Producer signatures were pooled across paired sites within each study region (n = 20 samples per algal group per region), while mean *Tegula* signatures were calculated separately for each site ($n_{site}$ = 50, N = 300). All grazer signatures were corrected for isotopic fractionation using a trophic enrichment factor of 1.5‰ for $\delta^{13}$C and 3.4‰ for $\delta^{15}$N. Bayesian mixing model (SIAR) estimates of macroalgal contribution to *Tegula* grazer diet at paired predator present (SS+) / absent (SS-) sites in each study region. Shaded boxes represent 50%, 75% and 95% credibility intervals for algal contribution estimates within a given study site, based on n= 50 individual snails sampled per location.

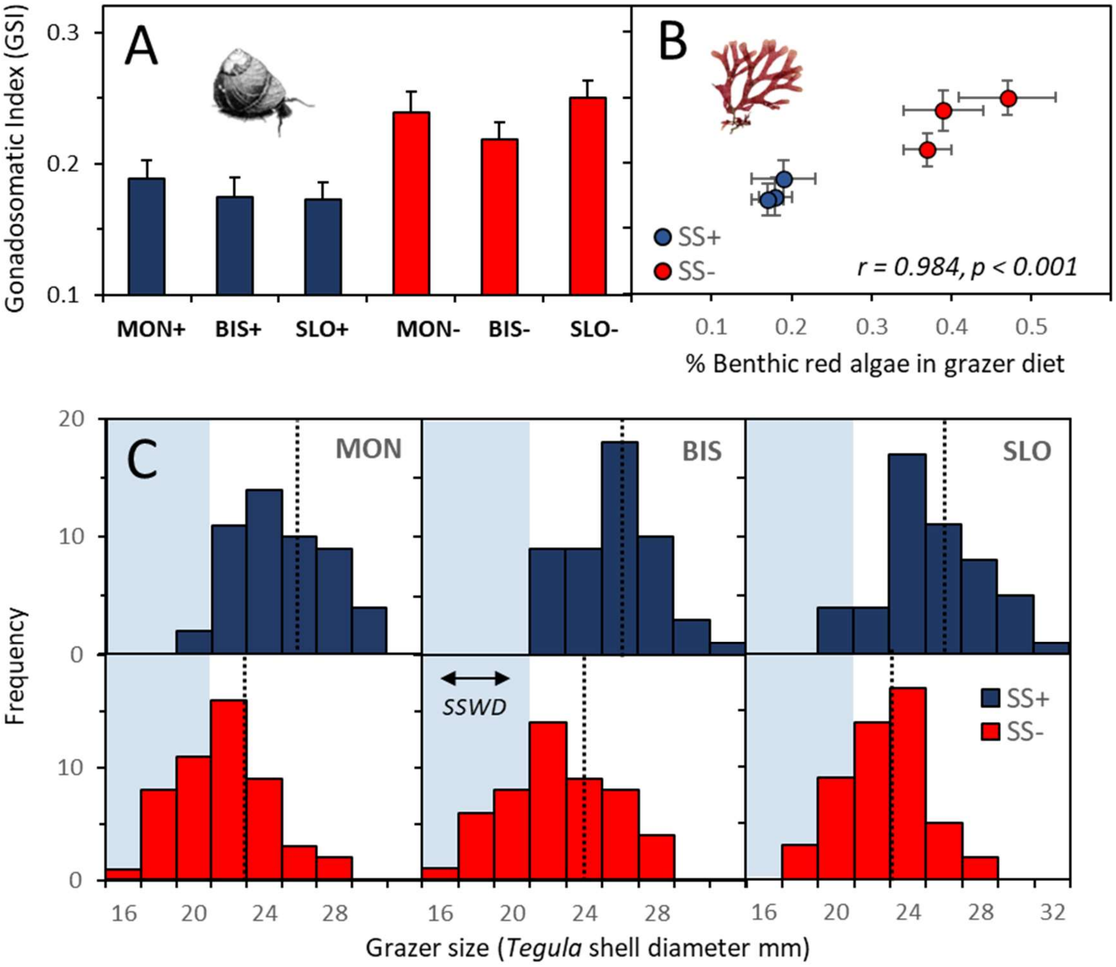


**Figure 3.** (A) Mean (± SE) gonadosomatic index [GSI] of individual *Tegula* grazers collected from predator present (+) and absent (-) sites in each study region (n = 50, total N = 300), and (B) population-scale relationship between grazer reproductive investment (GSI) and estimated dietary contribution of *M. pyrifera* across the six study sites. (C) Size frequency distributions of grazers (*Tegula* spp.) at paired SS+/SS- study sites in each of the three study regions (MON, BIS, SLO), with areas shaded in blue representing size classes estimated to be newly recruited post-disease (SSWD) outbreak.

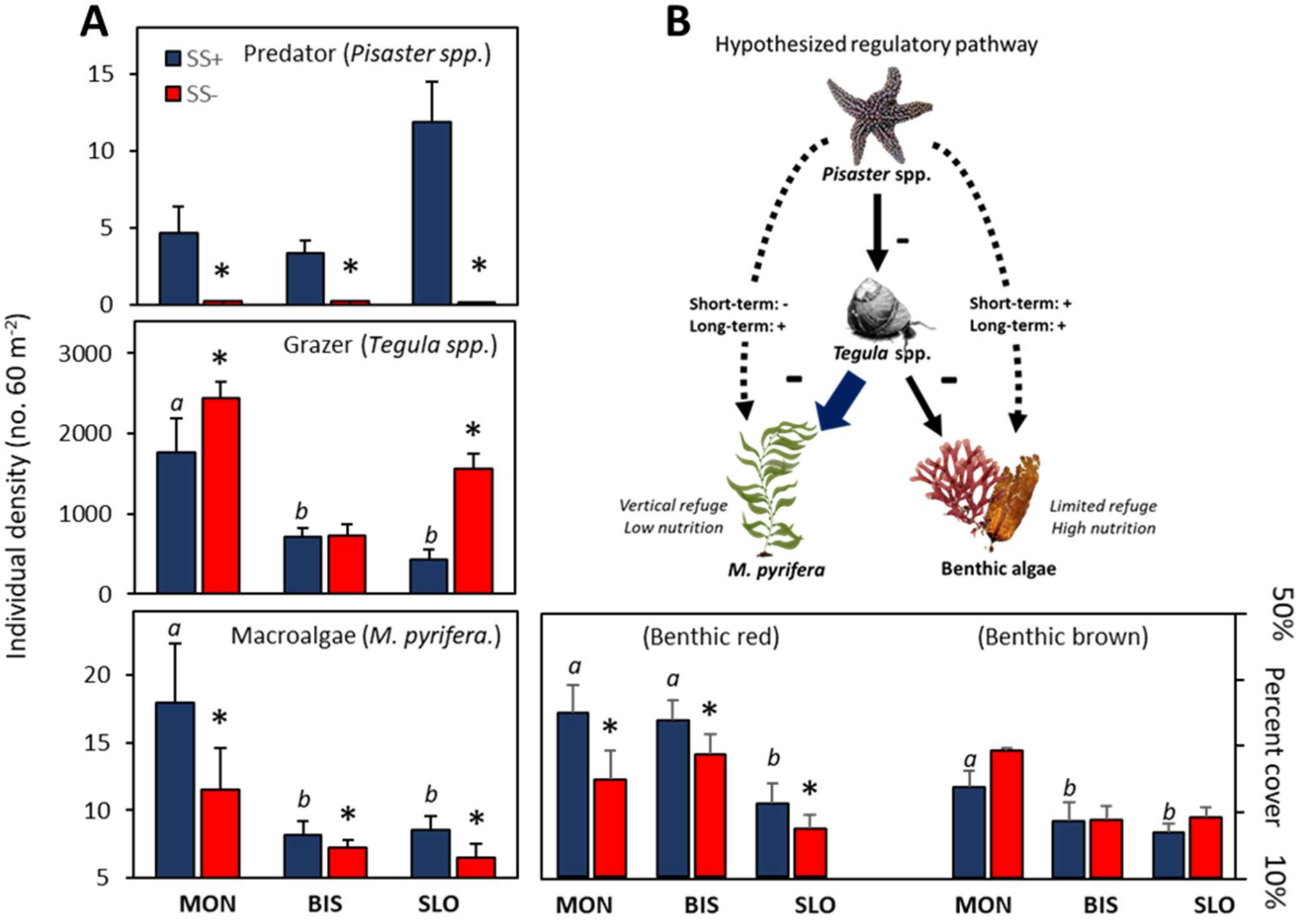


**Figure 4.** (A) Relative abundance of predatory sea stars (*Pisaster* spp.), grazers (*Tegula* spp.), and macroalgal producer groups (*M. pyrifera*¸ benthic red algae, benthic brown algae) observed at paired predator present/absent (SS+/SS-) sites during field surveys, compared to (B) the hypothesized net regulatory effect(s) of predatory sea star presence on *Tegula* grazing activity. Bar graphs (A) represent either mean transect densities or percent cover (± SE) for each study site, and asterisks denote significant differences between paired sites within a given study region (Monterey [MON], Big Sur [BIS], San Luis Obispo [SLO]).

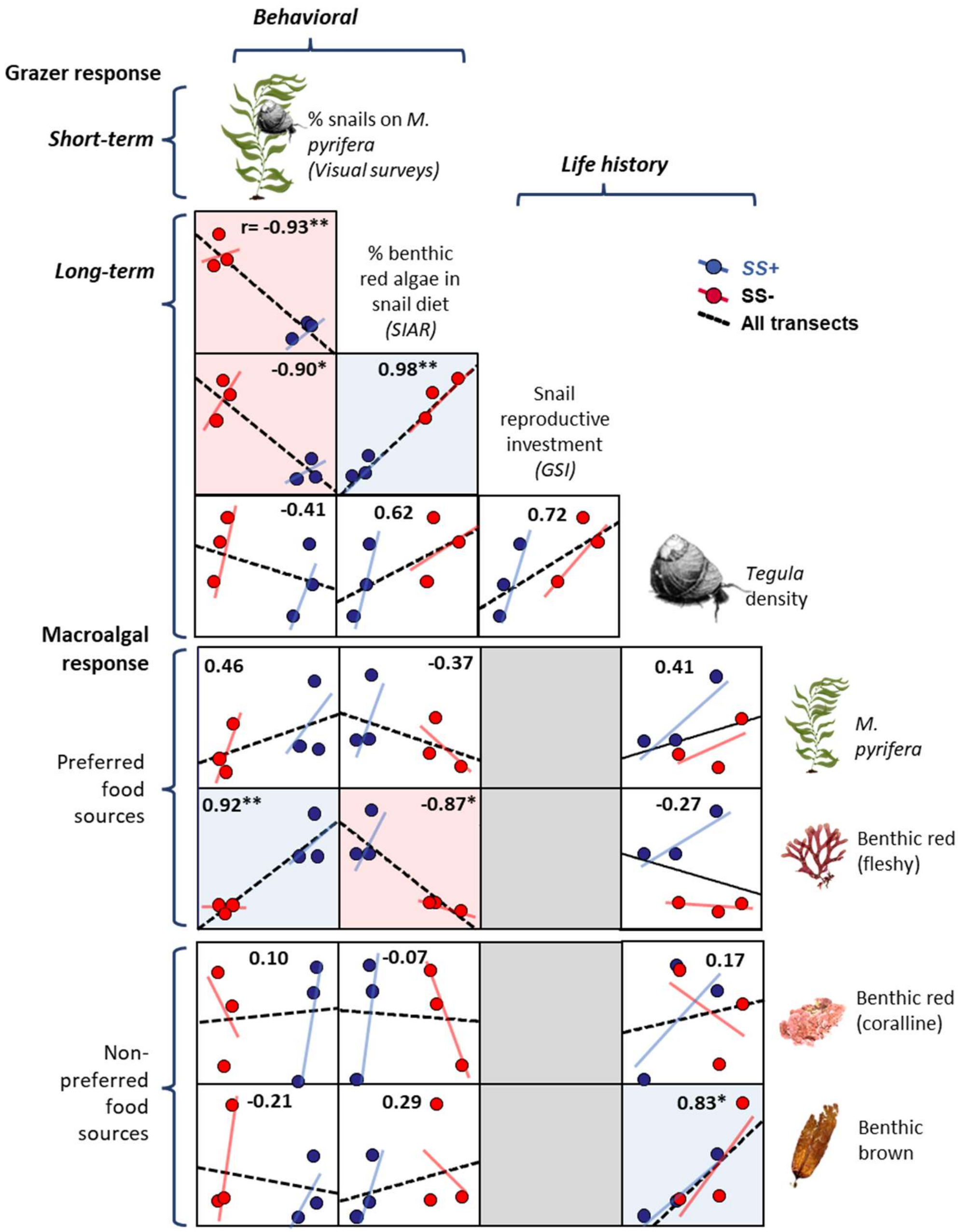


**Figure 5.** Correlation matrix depicting the strength and direction of reef- (site) scale relationships between observed short-term (behavioral, dietary) / long term (life history, reproductive) responses of *Tegula* grazers to predatory sea star release and local abundance of preferred and non-preferred macroalgal producers at predator present (SS+) and absent (SS-) sites (N = 6). Macroalgal abundance values for *M. pyrifera* represent the mean transect density for all replicates conducted within a given site (individuals/ 60m$^2$), and abundance values for benthic algal producer groups represent mean percent cover based on quadrat image analysis.

# CHAPTER 2

# Differential phenotypic responses by gastropods to large-scale disease-driven removal of sea star predators

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## ABSTRACT

The potential for aquatic gastropods to display phenotypic plasticity in response to predator cues is well documented in experimental settings. However, long-term physiological responses to predator exposure are difficult to evaluate at a large scale in the field. Thus, it is currently unclear (a) the extent to which comparatively dilute predator cues experienced by natural snail populations influence morphometric development and (b) if energetic costs associated with defensive morphology have allometric impacts on other life history characteristics (e.g., reproduction). The 2013 outbreak of sea star wasting disease in central California provided unique framework for a large-scale natural predator removal experiment, comparing shell morphometrics and gonadosomatic index (GSI) of subtidal *Tegula* turban snail populations at kelp forest sites where local predatory sea stars were completely absent (SS-) with paired sites maintaining low predator densities (SS+). All three snail species examined displayed significantly higher proportional allocation to shell mass at SS+ locations and concomitantly higher reproductive allocation with predators absent (SS-). Dietary stable isotope ($\delta^{13}C$, $\delta^{15}N$) analysis suggests this may be partially an energetic consequence of observed behavioral grazing shifts displayed by snails following predator release. Interestingly, observed shifts in shell morphometry varied among the three *Tegula* species, and appeared closely related to species-specific predator avoidance strategies.

***Key words:*** *TMII, inducible defense, sea star wasting disease, phenotypic plasticity, stable isotopes, GSI*

## INTRODUCTION

Phenotypic plasticity is the process by which organisms sharing a single, common genotype express multiple physical forms or life-history characteristics in response to variable external conditions (Bourdeau et al. 2012). Sources of external selection pressure can consist of abiotic, environmental, or physiological stressors (Fox et al. 2018) or biotic interactions (e.g., competition, predation; Kishida & Nishimura 2004, Lonnstedt et al. 2003). In many cases, morphological shifts observed in natural populations are influenced by a combination of these external factors (Ahlgren et al. 2013). For example, in terrestrial plant communities, a number of studies conducted in transitional ecosystems (e.g., salt marsh boundaries, montane slopes) have documented strong shifts in allometric growth, leaf morphology, and production of photosynthetic pigments in response to ecotonal gradients in light intensity, temperature, and hydrodynamic conditions (Atkin et al. 2006, Richards et al. 2010). In ecosystems where environmental variability is relatively low (e.g., deserts, rainforests), however, biotic interactions may be stronger drivers of phenotypic expression (Callaway et al. 2003). For example, plants commonly shift growth patterns (root: shoot allocation) when competitors are present to maximize uptake of limited resources (light, water, nutrients) or develop inducible defense mechanisms such as thorny structures (Young 1987, reviewed by Harvell 1990) and chemical compounds (Zangerl & Rutledge 1996) as deterrents to herbivory. Similar qualitative patterns have been observed for algal and invertebrate communities in marine intertidal ecosystems. Phenotypic plasticity in the upper intertidal zone is driven primarily by physiological stressors such as temperature (e.g., expression of heat shock proteins in bivalves; Berger & Emlet 2007) and evaporation (e.g., desiccation resistant turf growth forms in red algae; Taylor & Hay 1984), while phenotypic expression in the lower intertidal is driven primarily by biotic processes (i.e., competition for space or resources and predation threat; Brookes 2007, Flynn & Smee 2010) .

Because phenotypic plasticity can increase physiological tolerance to a wide range of environmental conditions and may provide an advantage in antagonistic biotic interactions (competition, predation), organisms with high capacity for phenotypic variability are often assumed to have increased fitness at both an individual and population level (Fox et al. 2019). For example, in tropical reef ecosystems, high levels of thermal plasticity in gene expression and metabolism displayed by some species of hermatypic corals have been cited as potential mechanisms to mitigate impacts of climate change on coral reefs (Sawall et al. 2015, Kenkel & Matz 2016). However, phenotypic variation in response to changing environmental conditions can result from either regulated or adaptive plasticity (in which organisms display integrated physical or behavioral modifications to optimize fitness in the presence of a specific environmental cue) or non-adaptive plasticity (in which altered life history characteristics such as decreased growth/reproduction are simply an indirect metabolic byproduct of sub-optimal environmental conditions; Merila & Hendry 2013, Bourdeau et al. 2012). Although non-adaptive plastic responses can increase the probability of individual survival by minimizing energy expenditure under conditions of resource limitation or high physiological stress, they may actually result in long-term reductions in fitness at a population level if decreasing investment in reproductive allocation significantly reduces population growth. Even when phenotypic plasticity occurs through an adaptive response, shifts in morphological development are often associated with substantial energetic costs and may result in fitness tradeoffs when multiple environmental stressors are present. In freshwater systems, for example, translucent-shelled aquatic snails (*Radix spp.*) can undergo multiple phenotypic shifts in mantle pigmentation in response to different environmental stressors, displaying darkened coloration to maximize photoprotection in response to UV radiation but increasing pattern complexity to maximize crypsis in response to visual predators. When both environmental stressors were present, snails retained a darker color morph with lower pattern complexity, suggesting an adaptive response that optimizes photoprotection at the expense of predation threat (Ahlgren et al. 2013).

Within marine systems, environmental variability in subtidal and low-intertidal habitats is typically less than variability in terrestrial and freshwater habitats, and there is substantial evidence that population regulation for upper-level consumers is maintained primarily through top-down biotic interactions (i.e., predation) rather than environmental stressors or resource limitation (Heck & Valentine 2007). Consequently, most examples of adaptive phenotypic plasticity documented in marine invertebrates and fishes have optimized defensive morphological adaptations in response to predation threat (Lonnstedt et al. 2003, Trussell & Smith 2000). Evidence for this inducible defense response is particularly prevalent in marine gastropods; multiple laboratory experiments conducted on a number of different species have commonly documented greater allocation to defensive growth (i.e., shell deposition rate) and substantial shifts in defensive morphology (i.e., shell deposition pattern) when juvenile snails are exposed to predator cues during early development (Bourdeau et al. 2012 and citations therein). However, the prevalence and magnitude of predator-induced morphological shifts for gastropods in natural settings (where predator cues are much more dilute than in most laboratory experiments) is still relatively unknown (Dewitt et al. 1998). Additionally, although laboratory experiments have demonstrated increased short-term fitness (i.e., lower predator-induced mortality) for individuals displaying defensive phenotypes or morphs, the long-term energetic consequences and potential life-history tradeoffs of maintaining defensive shell morphology through later (adult) life stages has not been quantified. Because of this, the relative importance of adaptive phenotypic plasticity in regulating population dynamics of wild gastropods remains unclear.

One of the major obstacles in evaluating population-level impacts of inducible defense response in subtidal marine gastropod populations is the inherent difficulty in monitoring or maintaining long-term predator exposure treatments in the field, where large-scale predator removal is often unviable from both an ethical and logistical standpoint. However, the 2013 outbreak of sea star wasting disease along the California coast provided a unique natural experimental system in which to evaluate population level impacts of predator-induced adaptive plasticity on an ecologically relevant, reef-wide scale that is not otherwise achievable with conventional manipulative studies. We selected subtidal turban snails (*Tegula spp.*) as a model organism for this research based on their abundance throughout the study regions, potential ecological importance as algal grazers (Sala and Graham 2002), and strong association with predatory sea stars (*Pisaster* spp*., Pycnopodia helianthoides*), which account for > 90% of predator-induced mortality in subtidal *Tegula* populations (Watanabe 1983a,b). By developing a paired sampling design comparing the physical characteristics of wild *Tegula* collected from sea star +/- sites in multiple study regions, we were able to isolate the effects of predator presence on *Tegula* allometric growth patterns (defensive vs. reproductive investment) and defensive morphology on both a local population- and individual level. Additionally, by comparing relative reproductive (gonad) and defensive (shell) investment to snail diet using stable isotopes, we were able to evaluate the likelihood that any observed allometric shifts to disease-induced predator removal were driven by passive energetic consequences vs. an adaptive response to perceived predation threat (i.e., inducible defense).

# METHODS

## *Study design and field collections*

In order to evaluate potential phenotypic responses of *Tegula* spp. to the presence of their dominant sea star predators, we utilized the 2013 outbreak of sea star wasting disease along the California coast as a natural experiment to compare allometric growth and shell morphometry of wild *Tegula* populations with differing histories of predator exposure. Over the time period that we conducted our field research (2015-2016), disease occurrence on subtidal reefs throughout the central California coast was regionally widespread but remained locally patchy, such that sites with severe wasting outbreaks (80-100% sea star loss) were often directly adjacent to sites maintaining moderate sea star densities. This spatial configuration allowed us to develop a paired sampling design directly comparing the effects of predator presence between reefs that were otherwise similar.

We selected sets of paired sites with sea stars present (SS+) or absent (SS-) in three study areas: Monterey (MON), Big Sur (BIS), and San Luis Obispo (SLO; Figure 1A). Initial site selection within each study area was conducted based on suitable rocky-reef, kelp-forest habitat, prevalence of sea star wasting disease (http://data.piscoweb.org/marine1/seastardisease.html), occurrence of proximate low disease impact (SS+) and high disease impact (SS-) reefs, and availability of baseline data on sea star density in the years leading up to the 2013 disease outbreak (https://www.piscoweb.org/find-pisco-dataone). Within each of the three study regions, distance between paired predator present (SS+) and absent (SS-) sites ranged from 2-8 km. Prior to field sample collections, preliminary visual surveys were conducted on SCUBA at each potential study site to quantify initial densities of sea star predators (Figure 1B) and confirm that paired SS+/- reefs were qualitatively comparable in depth, habitat structure, wave exposure, and algal communities. To document potential behavioral grazing shifts displayed by snails following predator removal, vertical algal habitat distribution (physical position of snails on either giant kelp or benthic understory algae) was also recorded for all *Tegula* observed on transects to determine proportional utilization of *Macrocystis pyrifera* (giant kelp) at SS+ vs. SS- sites (Figure 1C).

Field collections were conducted from September 2015 to January 2016, and paired sites within each study area were sampled sequentially within a 7-d time frame. After initial visual surveys were completed, divers collected 50 adult *Tegula spp.* from subtidal kelp forests (depth: 8-12 m) at each of the six sites for a total sample size (N) of 300 individuals. Snails were collected haphazardly and included all three common subtidal *Tegula* species native to the central California coast (*T. brunnea, T. montereyi, T. pulligo*). Although each species was regularly observed in every study region, *T. brunnea* was numerically dominant on visual surveys at all six study sites and represented the majority of samples collected from every location (Table 1). Within each region, representative tissue samples were also collected from three algal producer groups (*M. pyrifera* and representative local brown and red benthic understory algae) to establish region-specific signatures for use in dietary stable isotope analysis (n = 20 per algal group per region, total n = 60 each for *M. pyrifera*, benthic red and benthic brown understory algae).

### ***Allometric growth***

In the laboratory, all snail specimens were manually dissected to separate hard shells from somatic and reproductive tissue. The sex and reproductive state (ripeness) of each individual was then determined visually based on gross gonad morphology and coloration;

female reproductive tissue for *Tegula spp.* is typically dark green in color, whereas male reproductive tissue is a creamy white (Ortiz-Ordonez et al. 2009).

Reproductive allocation is closely related to age class in marine gastropods (e.g., Kido and Murray 2003), but because local *Tegula* populations in central California do not consistently deposit the clear annual growth bands displayed by cooler water populations further north (Oregon-Washington, Cooper and Shanks 2011), we were not able to assign reliable age estimates to specimens collected from the study regions evaluated here. Thus, in order to minimize potential confounding effects of demography across sites, *Tegula* samples were limited to include only adult specimens measuring ≥ 15mm shell diameter. All individuals within this size range were designated as reproductively mature based on visual observations of gonad condition as well as the published size at maturity for a regionally abundant intertidal congener (*T. funebralis;* Paine 1971).

Following dissection, each shell sample was rinsed thoroughly in fresh water and scrubbed to remove encrusting organisms/debris from the exterior surface, then dried in an oven at 60 °C for a minimum of 24 h to remove residual moisture. Soft tissue (somatic and gonad) samples were dried separately at 60 °C for a minimum of 48 h. All samples were then weighed to the nearest 0.01 g, and dry-weight biomass estimates for shell material, somatic tissue, and gonad tissue were recorded for each individual.

Proportional allocation to reproductive tissue was evaluated across study sites using gonadosomatic index (GSI) and proportional allocation to defensive growth was evaluated using an index of shell biomass (SI) to total biomass. Calculations of allometric growth indices were based on the following equations:

$$GSI = \frac{Gonad\ Dry\ Weight}{Total\ Soft\ Tissue\ Dry\ Weight}\ x\ 100$$

$$SI = \frac{Shell\ Weight}{Total\ Dry\ Weight\ (Shell + Soft\ Tissue)}\ x\ 100$$

To evaluate potential energetic links between allometric growth allocation and observed shifts in snail grazing behavior at SS- sites during field surveys (see Figure 1C), dried tissue samples from both snails and potential algal food sources (giant kelp *Macrocystis*, benthic red algae, benthic brown algae) were first lipid-extracted to minimize potential anomalies in $\delta^{13}C$ signatures due to variable fat content, then analyzed for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility. Prior to isotopic analysis, snail tissue samples were manually dissected to isolated only somatic muscle tissue (with all gonad material excluded). Here, we present only $\delta^{13}C$ data for *Tegula* spp. and the two algal producer groups (*M. pyrifera,* benthic red algae) identified in (dual-isotope) SIAR mixing models to be dominant contributors to snail diet; $\delta^{15}N$ signatures in the current study were interpreted only in preliminary analyses to evaluate benthic brown understory algae as a significant food source. Full dietary stable isotope model results from each study site are evaluated more comprehensively in the previous chapter (Chapter 1).

### *Shell morphometry*

To evaluate potential shifts in defensive growth morphology between predator present (SS+) and absent (SS-) sites within each region, we conducted a series of six morphometric shell measurements (see Figure 2): shell height [SH], shell width [SW], spire [SP], aperture height [AH], aperture width [AW] and radius [RA]. Approximated shell volume for each individual was calculated using the measured radius value and the geometric formula for the volume of a cone ($V = 1/3\ h \cdot \pi r^2$). All measurements were taken with calipers to the nearest 0.1mm, and using these measurements, we calculated the following morphometric indices: shell shape (height: width ratio, SH/SW) , aperture shape (height: width ratio, AH/AW), shell volume: body tissue ratio (V/ total somatic and gonad tissue weight) and comparative shell thickness, calculated using the proxy of shell weight standardized by shell volume (shell weight/V). These particular metrics were selected to evaluate specific aspects of snail predator avoidance behavior previously documented for the subtidal *Tegula* species in the current study (see Watanabe 1984), as well as aquatic gastropods in other marine and freshwater systems (see McNair et al. 1981, Verhaegen et al. 2019).

### ***Data analysis***

For allometric growth analyses, gonadosomatic index (GSI) and shell index (SI) were analyzed separately for each *Tegula* species using a general linear model (GLM) approach, with region (MON, BIS, SLO) and predator presence (SS+ / SS-) as main effects. All four shell morphometry metrics of interest (total shell H / W, aperture H / W, shell volume / soft body tissue ratio, shell weight / shell volume ratio) were also analyzed separately by species using a similar GLM approach with study region and predator presence as main effects, and in cases where model results indicated a significant region x predator interaction, pairwise comparisons were conducted between paired SS+/SS- snail samples within each of the three regions to parse apart potential large scale regional gradients in snail morphology from potential effects of predator presence. All data were evaluated for homoscedasticity and normality prior to interpreting model results, and ln-transformed when necessary to satisfy assumptions of statistical tests.

Because the two algal groups that dominated snail diets (i.e., *M. pyrifera*, benthic red algae) displayed similar $\delta^{15}$N signatures but were clearly separated in $\delta^{13}$C (mean value of -14.5

[95% CI:-17.4 to -11.6] for *M. pyrifera vs.* -30.9 [95% CI:-34.4 to -27.4] for benthic red algae, pooled across regions), isotopic $\delta^{13}$C values of snail tissue were selected here as a proxy for benthic algal utilization to evaluate potential mechanistic relationships between snail behavioral grazing responses to predator presence (SS+/SS-) and energetic investment in allometric growth ( GSI and SI), as well as a potential driver of shell morphometry (see Maltseva et al. 2020). For each of the three *Tegula* species, potential relationships between individual $\delta^{13}$C signatures and reproductive investment (GSI) were evaluated using simple correlation analyses, run separately for SS+ and SS- snail samples, pooled across regions. To evaluate a potential link between *Tegula* diet and reproductive activity, mean tissue $\delta^{13}$C signatures were also compared between groups of snails displaying ripe vs. non-ripe gonads within SS+ and SS- samples. This was evaluated separately for each species using two-way analysis of variance (ANOVA), with gonad state (ripe, non-ripe) and predator presence (SS+, SS-) as main effects.

## RESULTS

### *Allometric growth and dietary signatures*

Shifts in allometric growth displayed by *Tegula* following predatory sea star removal appeared to be consistent across species as well as across the three study regions (Figure 3); *T. brunnea, T. montereyi,* and *T. pulligo* all displayed significantly greater proportional investment in reproductive tissue (GSI) and significantly less proportional investment in defensive shell growth (SI) at SS- sites compared to paired SS+ locations (GLM, $p < 0.05$) and no significant regional effects or region x predator interactions were detected for any of the three species (Table 2). Across all species and study regions, the proportion of individual snails displaying ripe gonads in samples was also consistently greater in the absence of predatory sea stars, with mean values ranging from 0.33- 0.60 at SS+ locations and 0.64 – 1.00 at SS- sites (see Table 1).

All three *Tegula* species displayed a significant effect of predator presence on tissue (dietary) $\delta^{13}C$ signatures (GLM, $p < 0.01$; Table 2), with observed isotopic values for SS- snail samples (mean: -20.5 to -24.5) consistently more depleted compared to SS+ collections (mean: -17.4 to -18.5; Table 3). Only *T. brunnea* displayed a significant regional effect and region x predator interaction; however, this was driven by an observed SS+/SS- shift in SLO that was greater in magnitude than at the other two study regions, rather than any difference in the directional effect of predatory sea star presence. Isotopic $\delta^{13}C$ signatures for all three snail species displayed greater variability in SS- samples compared to SS+ collections (Figure 4A, see also standard error estimates in Table 3), with values that tended to fall in the intermediate range between $\delta^{13}C$ signatures for giant kelp (*Macrocystis;* 95% CI:-17.4 to -11.6) and benthic red algae (95% CI:-34.4 to -27.4), compared to SS+ individuals which tended to cluster fairly tightly around the regional signature for *Macrocystis.*

For two of three *Tegula* species, stable isotope results from SS- samples revealed evidence that snail reproductive investment may be closely related to algal diet when predatory sea stars are removed; both *T. brunnea* and *T. pulligo* collected from SS- locations displayed a significant inverse relationship between individual snail GSI values and $\delta^{13}C$ signatures (Pearson correlation, $r= -0.39$, $p< 0.001$; $r= 0.66$, $p= 0.001$) indicating that greater consumption of benthic red algae (evidenced by a depleted, more negative $\delta^{13}C$ signature) may be associated with increased reproductive output (Figure 4A). *T. montereyi* displayed a similar qualitative trend, with generally greater GSI values and more depleted $\delta^{13}C$ signatures at SS- locations, but the relative rarity of this species in random samples limited power for statistical inference. No significant relationships between snail dietary $\delta^{13}C$ signatures and reproductive investment (GSI) were detected in the presence of predatory sea stars (i.e., SS+ sites), likely because snail dietary signatures at these locations displayed limited individual variability and consistently resembled *Macrocystis*, suggesting that benthic algal utilization when predators are present may be uniformly low.

Similarly, when $\delta^{13}C$ signatures were compared between snail samples displaying ripe (active egg/sperm development) vs. non-ripe (dormant) gonads, isotopic values for both *T. brunnea* and *T. pulligo* differed significantly by gonad state (ripe, non-ripe) as well as predator presence (SS+/SS-), with significantly more depleted values (i.e., more closely resembling benthic red algae) observed overall in ripe (i.e., reproductively active) individuals and predator absent locations (SS-; Figure 4B, Table 4). However, both species also displayed a significant interaction effect between gonad ripeness and predator presence; post-hoc pairwise comparisons revealed similar $\delta^{13}C$ signatures between ripe and non-ripe individuals at SS+ sites in the presence of predatory sea stars, but significantly depleted isotopic signatures in ripe individuals at SS- sites with predators removed. Again, *T. montereyi* showed qualitatively similar results (see Figure 4B), but for this species, only predator presence was statistically significant, likely due to limited sample size combined with the moderate individual variability observed here for $\delta^{13}C$ signatures in SS- samples.

### *Shell morphometry*

Unlike allometric growth and dietary analyses which were generally similar across the three *Tegula* species, observed shifts in shell deposition pattern and morphometry differed distinctly both qualitatively and quantitatively between *T. pulligo* and the other two taxa. Both *T. brunnea* and *T. montereyi* displayed a significant shift in shell shape based on predatory sea star presence (GLM, $p < 0.001$; Table 2), with consistently larger shell height: width ratios (i.e., taller, narrower shells) observed at paired SS+ sites in all three study regions (Figure 5A).

Concordantly, both of these species also displayed larger ratios of shell volume to soft body tissue mass at SS+ sites compared to SS- locations, although the effects of predator presence for this metric were highly significant for *T. brunnea* ($p < 0.001$) and only marginally significant for *T. montereyi* due to limited sample numbers (GLM, $p = 0.063$; Table 2).

In contrast, *T. pulligo* across all three study regions did not display any discernable difference in overall shell shape (height: width ratio) between SS+ and SS- locations (Figure 5A) and no significant effects of predator presence were detected for either shell height: width ($p = 0.687$) or shell volume: body mass (GLM, $p = 0.920$; Table 2). However, of the three species, only *T. pulligo* displayed a significant difference in shell density (shell weight/volume) between SS+ and SS- locations, which was calculated as a proxy for shell thickness to account for shell deposition strategies prioritizing internal reinforcement over outward (i.e., marginal) growth (Figure 5B). Here, shell density for *T. pulligo* was consistently higher in the presence of predatory sea stars (SS+) in all three study regions ($p < 0.001$) while both *T. brunnea* and *T. montereyi* failed to display a consistent or significant predator effect (GLM, $p = 0.207, 0.302$; Table 2).

Although overall shell growth and deposition patterns revealed substantial variability among species, all three species displayed some similar patterns of recent deposition, evaluated here as shell aperture shape (height: width ratio) between SS+/SS- sites. Snails in the presence of predatory sea stars consistently displayed rounder aperture shapes (i.e., significantly lower height: width ratio, typically $\approx 1.0$-$1.2$) at SS+ sites (GLM, $p < 0.001$ for all three species; Table 2), compared to more oblong (higher height: width) aperture shapes ($\approx 1.4$ -$1.6$ ratio) for snails at SS- sites.

# DISCUSSION

Although evidence for predator-induced phenotypic plasticity in aquatic gastropods has been widely demonstrated in laboratory settings, the ecological significance and long-term fitness consequences of these inducible defenses have often proved difficult to evaluate at a large scale in the field. This has been particularly true in marine subtidal systems such as temperate kelp forests and rocky reefs, where long-term manipulations of predator density are often unfeasible, and many species of gastropods are relatively mobile, making it difficult to monitor predator exposure and track long-term physiological responses of individual snails. Here, the 2013 outbreak of sea star wasting disease along the central California coast provided a unique opportunity to evaluate the prevalence and magnitude of predator-induced phenotypic plasticity for wild populations of three congeneric gastropod species (*Tegula brunnea, T. montereyi, T. pulligo*) commonly found throughout subtidal kelp forests in the region. Because disease-driven local extirpations of predatory sea stars (*Pisaster* spp., *Pycnopodia helianthoides*) typically occurred on a relatively large, reef-wide scale and persisted for several years prior to sampling, we were able to compare morphological and life history characteristics of local snail populations with a history of recent predator exposure (i.e. sites where sea stars were not completely extirpated by disease) to nearby snail populations that had experienced long-term predation release over multiple reproductive seasons. This revealed substantial evidence that marine gastropods in subtidal systems may have widespread capability to alter growth patterns and life history strategies in response to local predator presence at a population level.

In terms of allometric growth, all three species of *Tegula* displayed consistently greater proportional allocation to reproductive investment (i.e., gonad tissue) and lesser allocation to defensive growth (shell deposition) at sites where predatory sea stars were locally absent (SS-)

compared to paired (SS+) locations, and qualitative results were consistent across all three regions (Monterey, Big Sur, San Luis Obispo). Similar shifts in life-history strategy (i.e., growth allocation and reproduction) as a response to predator presence have been well documented for marine and freshwater gastropods, albeit in laboratory settings. Multiple studies conducted on juvenile snails have documented significant increases in shell thickness for individuals exposed to chemical cues from predators and/or crushed conspecifics during early development (Trussell 1996; Dalziel & Boulding 2005; Auld & Relyea 2011), and increased investment in shell deposition has often been associated with fitness tradeoffs in other areas, such as decreased size and somatic growth (Trussell 2006; Trussell and Nicklin 2002). Adult snails have also been shown to display earlier onset of reproductive maturity when exposed to predator cues in laboratory settings, accompanied by slower growth rates and lower fecundity compared to snails maintained in control tanks without a predator cue (Guo et al. 2017). On an individual scale, observed relationships between depleted *Tegula* $\delta^{13}$C signatures (indicating benthic red algae in diet) and reproductive investment (GSI, gonad ripeness) further suggest that increased reproductive allocation at SS- sites may be a passive energetic consequence of predatory grazer release and increased access to nutritionally superior benthic food sources, which is similar to tradeoffs between optimal foraging and predator avoidance documented in a number of other systems (e.g., Holbrook and Schmitt 1988, reviewed in Lind and Cresswell 2005, Creel 2018).

However, although the observed shifts in allometric growth displayed by *Tegula* spp. in the current study are qualitatively similar in many ways to predator responses documented in previous studies, the results here are particularly notable because they occurred in wild populations in a marine, subtidal system where predator chemical cues are likely to be far more dilute compared to experiments conducted in controlled mesocosms (see Haggerty et al. 2018), or even the few long-term field experiments conducted in enclosed tidepools (e.g. Trussell 2006). This is particularly true for the time period that we conducted our field surveys in that predatory sea star densities, even at our SS+ sites, were almost an order of magnitude below historical levels, with an average of only 4-12 individuals observed on 60-$m^2$ transects (Figure 1). That there were consistent and significant differences in allometric growth and reproductive allocation between paired SS+/- sites despite the low abundance of predators at SS+ locations suggests that top-down processes may play a substantial role in influencing life-history strategies and regulating population dynamics for gastropods in this system.

Although all three subtidal *Tegula* species examined in the current study displayed similar increases in shell investment at sites with predatory sea stars present (SS+) compared to paired SS- absent sites where predatory *Pisaster* and *Pycnopodia* were completely absent locally, results of more detailed shell morphometric analysis suggest that observed shifts in overall defensive investment are driven by different structural or physiological processes across species, and these appeared to be closely related to species-specific predator avoidance strategies. Across all three sets of paired study sites, *T. brunnea* and *T. montereyi* displayed significant decreases in the ratio of shell height to width at sites where predators were absent, while *T. pulligo* displayed a significantly greater ratio of height to width when predatory sea stars were removed (Figure 4). Both *T. brunnea* and *T. montereyi* utilize flight as a primary defense against sea star predation, followed by evasive maneuvers (i.e., detaching from the substratum) or withdrawing into the shell as a secondary strategy if fleeing is unsuccessful (Watanabe 1983a,b). Given the secondary defensive response displayed by both of these species combined with the feeding mechanics of *Pisaster* and *Pycnopodia* as suction predators, it is reasonable that a taller, narrower shell would provide greater protection for these two species, and thus be adaptive at SS+ locations where snails still perceive predation threat. However, *T. pulligo* is unique in that it clamps down on the substrate rather than fleeing as a primary defense strategy against sea star predation (Watanabe 1983), and thus, it makes sense that this species already displays a flatter, wider shell (i.e., increased surface area for substrate attachment) at sites where predator cues are perceived and would likely receive greater fitness advantage from increasing shell weight and thickness to further prevent dislodgment.

Results presented here suggest that marine gastropods in subtidal systems may have widespread capability to alter growth patterns and life history strategies in response to predator presence; experimental *Tegula* populations for all three species examined displayed consistently greater proportional allocation to reproductive investment (i.e. gonad tissue) at sites where predatory sea stars were locally absent (SS-) compared to paired (SS+) sites, as well as significant differences in patterns of recent shell growth likely corresponding to the time period after disease outbreak (i.e., pertaining to deposition zones internally and around the aperture; e.g., Larsson et al. 2020). Because disease-driven local extinctions of predatory sea stars typically occurred on a relatively large, reef-wide scale, we were able to determine that snails exposed to long-term predator release did in fact display significant shifts in life history strategy in terms of both defensive morphology (i.e., phenotypic plasticity) and reproductive investment. The consistent life history shifts displayed at all three SS- sites suggest that predatory sea stars can play a critical role in regulating local population dynamics for subtidal *Tegula* in the region, and if so, future disease outbreaks or other anthropogenic disturbances causing large-scale predator removal could have significant ecological impacts in disrupting top-down regulation of grazers in this system. Given that many coastal ecosystems are thought to be strongly regulated by top-down processes, characterizing population-level effects of predator presence on lower-level grazers such as gastropods is a critical component to understanding the processes maintaining productivity and stability within coastal food webs.

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# TABLES

**Table 1.** Summary of *Tegula* samples, by species, haphazardly collected from paired predatory sea star present (SS+) vs. absent (SS-) sites in each of the three study regions (total n = 50 snails per site, total n = 300). Sex ratio is presented as the proportion of females within a given sample (F:M), and gonad ripeness (% ripe) represents the proportion of gravid individuals in the sample based on visual inspection.

| | | *T. brunnea* | | | *T. montereyi* | | | *T. pulligo* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | n | Sex ratio | % ripe | n | Sex ratio | % ripe | n | Sex ratio | % ripe |
| Monterey | SS+ | 33 | 0.55 | 0.52 | 9 | 0.56 | 0.44 | 8 | 0.50 | 0.50 |
| | SS- | 32 | 0.50 | 0.75 | 7 | 0.57 | 0.86 | 11 | 0.55 | 0.64 |
| | **Total** | **65** | | | **16** | | | **19** | | |
| Big Sur | SS+ | 34 | 0.53 | 0.50 | 6 | 0.50 | 0.33 | 10 | 0.60 | 0.60 |
| | SS- | 38 | 0.53 | 0.71 | 5 | 0.60 | 0.80 | 7 | 0.57 | 0.86 |
| | **Total** | **72** | | | **11** | | | **17** | | |
| San Luis Obispo | SS+ | 40 | 0.48 | 0.50 | 3 | 0.67 | 0.33 | 7 | 0.43 | 0.57 |
| | SS- | 44 | 0.52 | 0.80 | 1 | 0.00 | 1.00 | 5 | 0.40 | 1.00 |
| | **Total** | **84** | | | **4** | | | **12** | | |
| **Grand Total** | | **221** | | | **31** | | | **48** | | |

**Table 2.** Summary of general linear model (GLM) results for reproductive (gonad) and defensive (shell) allometric growth and dietary δ13C signature, as well as morphometric shell indices, with significant effects highlighted in bold. For all models where predator presence was found to have a significant effect on snail morphology, the observed directional shift between SS+ and SS- sites is indicated in italics below.

| Allometric investment | | *T. brunnea* | | | *T. montereyi* | | | *T. pulligo* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Source | d.f. | *F*-value | *P* | d.f. | *F*-value | *P* | d.f. | *F*-value | *P* |
| Gonadosomatic Index (GSI) | *Predator* | **1** | **15.48** | **< 0.001** | **1** | **7.01** | **0.014** | **1** | **4.26** | **0.045** |
| | *Region* | 2 | 1.12 | 0.33 | 2 | 0.00 | 0.996 | 2 | 0.14 | 0.867 |
| | *P x R* | 2 | 0.45 | 0.64 | 2 | 0.91 | 0.414 | 2 | 0.91 | 0.409 |
| | | | ***SS+ < SS-*** | | | ***SS+ < SS-*** | | | ***SS+ < SS-*** | |
| Shell Index (SI) | *Predator* | **1** | **45.75** | **< 0.001** | **1** | **8.21** | **0.008** | **1** | **20.41** | **< 0.001** |
| | *Region* | **2** | **17.96** | **< 0.001** | 2 | 2.00 | 0.156 | 2 | 0.18 | 0.834 |
| | *P x R* | **2** | **5.90** | **0.003** | 2 | 0.56 | 0.58 | **2** | **3.34** | **0.042** |
| | | | ***SS+ > SS-*** | | | ***SS+ > SS-*** | | | ***SS+ > SS-*** | |
| Dietary tissue δ13C | *Predator* | **1** | **60.88** | **< 0.001** | **1** | **13.75** | **0.001** | **1** | **18.39** | **< 0.001** |
| | *Region* | **2** | **5.55** | **0.004** | 2 | 0.39 | 0.682 | 2 | 2.31 | 0.112 |
| | *P x R* | **2** | **5.10** | **0.007** | 2 | 0.32 | 0.726 | 2 | 0.85 | 0.436 |
| | | | ***SS+ > SS-*** | | | ***SS+ > SS-*** | | | ***SS+ > SS-*** | |
| Shell morphometry | | *T. brunnea* | | | *T. montereyi* | | | *T. pulligo* | | |
| | *Source* | d.f. | *F*-value | *P* | d.f. | *F*-value | *P* | d.f. | *F*-value | *P* |
| *Shell shape (H:W)* | *Predator* | **1** | **63.99** | **< 0.001** | **1** | **17.72** | **< 0.001** | 1 | 0.17 | 0.687 |
| | *Region* | **2** | **9.03** | **< 0.001** | 2 | 1.19 | 0.321 | 2 | 0.78 | 0.465 |
| | *P x R* | **2** | **4.49** | **0.012** | 2 | 2.08 | 0.146 | 2 | 0.01 | 0.986 |
| | | | ***SS+ > SS-*** | | | ***SS+ > SS-*** | | | --- | |
| *Shell density (mass: volume)* | *Predator* | 1 | 1.60 | 0.207 | 1 | 1.11 | 0.302 | **1** | **18.06** | **< 0.001** |
| | *Region* | **2** | **7.44** | **0.001** | 2 | 1.71 | 0.201 | **2** | **5.81** | **0.006** |
| | *P x R* | **2** | **8.34** | **< 0.001** | 2 | 0.37 | 0.698 | 2 | 0.08 | 0.925 |
| | | | --- | | | --- | | | ***SS+ > SS-*** | |
| *Shell volume: Body tissue mass* | *Predator* | **1** | **17.53** | **< 0.001** | **1** | **3.77** | **0.063** | **1** | 0.01 | 0.92 |
| | *Region* | **2** | **3.68** | **0.027** | 2 | 1.30 | 0.289 | **2** | 1.64 | 0.21 |
| | *P x R* | 2 | 7.45 | 0.001 | 2 | 1.84 | 0.179 | 2 | 1.67 | 0.20 |
| | | | ***SS+ > SS-*** | | | ***SS+ > SS-*** | | | --- | |
| *Aperture shape (H:W)* | *Predator* | **1** | **488.21** | **< 0.001** | **1** | **51.86** | **< 0.001** | **1** | **67.16** | **< 0.001** |
| | *Region* | 2 | 0.94 | 0.392 | **2** | **3.59** | **0.043** | **2** | 0.32 | 0.728 |
| | *P x R* | 2 | 0.43 | 0.649 | 2 | 0.27 | 0.768 | 2 | 0.27 | 0.762 |
| | | | ***SS+ < SS-*** | | | ***SS+ < SS-*** | | | ***SS+ < SS-*** | |

**Table 3.** Summary of mean δ13C signatures (± SE) for each of the three *Tegula* species by study region and predator presence (SS+/SS), as well as empirically derived δ13C signatures for the two main algal producer groups comprising *Tegula* diet (*Macrocystis*, benthic red algae). Algal signatures represent pooled values from all samples taken across the three study regions (n = 60 per taxa), snail signatures represent corrected values, adjusted for isotopic fractionation using a trophic enrichment factor of 1.5‰.

| **Species** | **Region** | **δ13C (SS+)** | **δ13C (SS-)** |
|---|---|---|---|
| *T. brunnea* | *MON* | -17.7 ± 0.1 | -20.6 ± 0.7 |
| | *BIS* | -18.1 ± 1.0 | -20.7 ± 0.9 |
| | *SLO* | -18.0 ± 0.4 | 23.7 ± 1.0 |
| *T. montereyi* | *MON* | -17.4 ± 0.27 | -21.0 ± 1.3 |
| | *BIS* | -18.1 ± 0.4 | -20.7 ± 0.9 |
| | *SLO* | -18.2 ± 0.9 | -21.0 |
| *T. pulligo* | *MON* | -17.6 ± 0.2 | -21.0 ± 1.6 |
| | *BIS* | -17.5 ± 0.5 | -20.5 ± 0.4 |
| | *SLO* | -18.5 ± 0.5 | -24.5 ± 2.3 |

| **Algal food sources** | **δ13C (Mean)** | **95% CI** |
|---|---|---|
| δ13C (Macrocystis) | -14.5 | -17.4 to -11.6 |
| δ13C (Benthic red algae) | -30.9 | -34.4 to -27.4 |

**Table 4.** Summary of general linear model (GLM) results evaluating potential relationships between *Tegula* diet (based on snail δ13C signatures) and gonad state (ripe vs. non-ripe) in the presence and absence of predatory sea stars (SS+/SS-), pooled across study regions. For model results indicating a significant interaction between gonad state and predator presence, results of post-hoc pairwise comparisons (1-way ANOVA) run separately for SS+ and SS- samples are also included below.

| Dietary tissue | δC | *Complete model* | | | *Pairwise by predator presence* | | | |
|---|---|---|---|---|---|---|---|---|
| Species | *Effect* | d.f. | *F* -value | *P* | | d.f. | *F* -value | *P* |
| *T. brunnea* | *Gonad ripeness* | 1 | 150.54 | < **0.001** | | | | |
| | *Predator* | 1 | 33.84 | < **0.001** | SS+ | 1 | 0.01 | 0.913 |
| | *G x P* | 1 | 12.18 | **0.001** | SS- | 1 | 12.02 | **0.001** |
| *T. montereyi* | *Gonad ripeness* | 1 | 1.39 | 0.249 | SS+ | --- | --- | --- |
| | *Predator* | 1 | 8.66 | **0.007** | SS- | --- | --- | --- |
| | *G x P* | 1 | 0.54 | **0.47** | | | | |
| *T. pulligo* | *Gonad ripeness* | 1 | 13.498 | **0.001** | SS+ | 1 | 0.41 | 0.842 |
| | *Predator* | 1 | 4.93 | **0.032** | SS- | 1 | 11.82 | **0.002** |
| | *G x P* | 1 | 12.56 | **0.001** | | | | |

# FIGURES

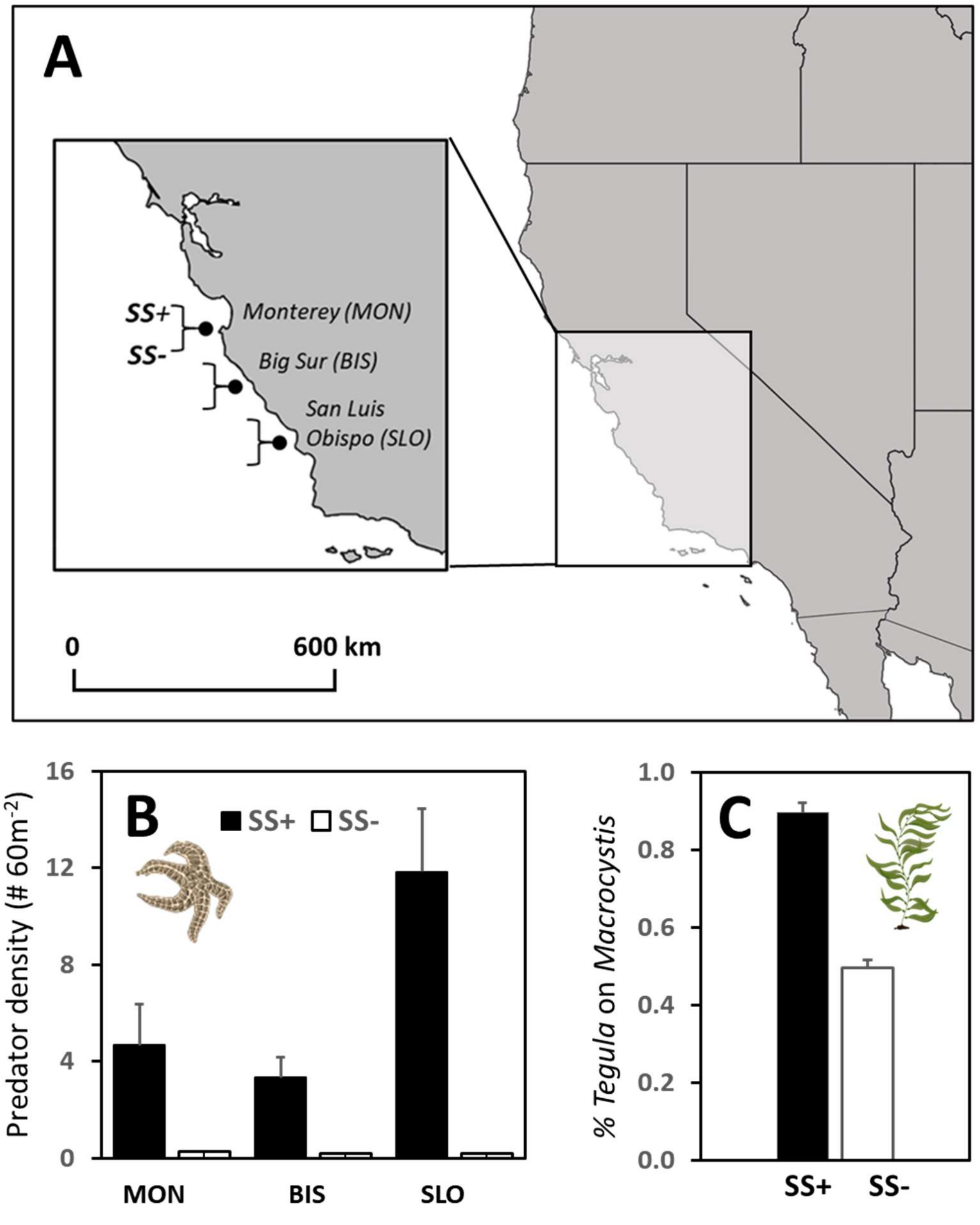

**Figure 1.** (A) Map of paired predator present (SS+) / absent (SS-) study sites in each of three study regions, (B) mean (± SE) densities of predatory sea stars (*Pisaster* spp.) observed on transects within each region, and (C) observed behavioral shift in vertical habitat utilization of *Tegula* spp., based on mean transect proportions of snails observed on giant kelp *Macrocystis pyrifera* in the presence (SS+) and absence (SS-) of predators, pooled across regions.

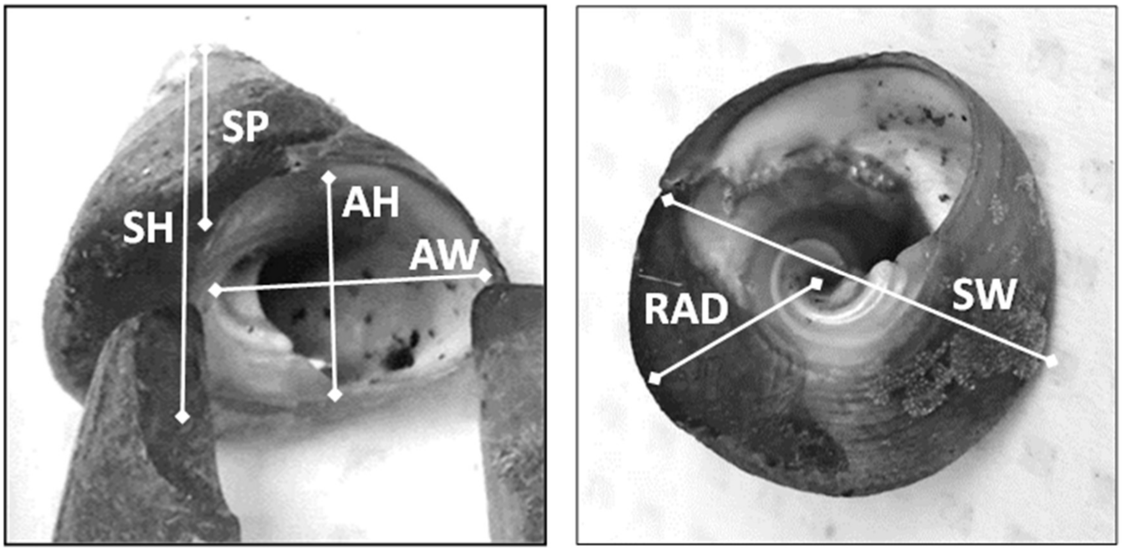

**Figure 2.** Summary of physical measurements taken on shell specimens for morphometric analysis: shell height [SH], shell width [SW], aperture height [AW], aperture width [AW], spire [SP], radius [RAD].

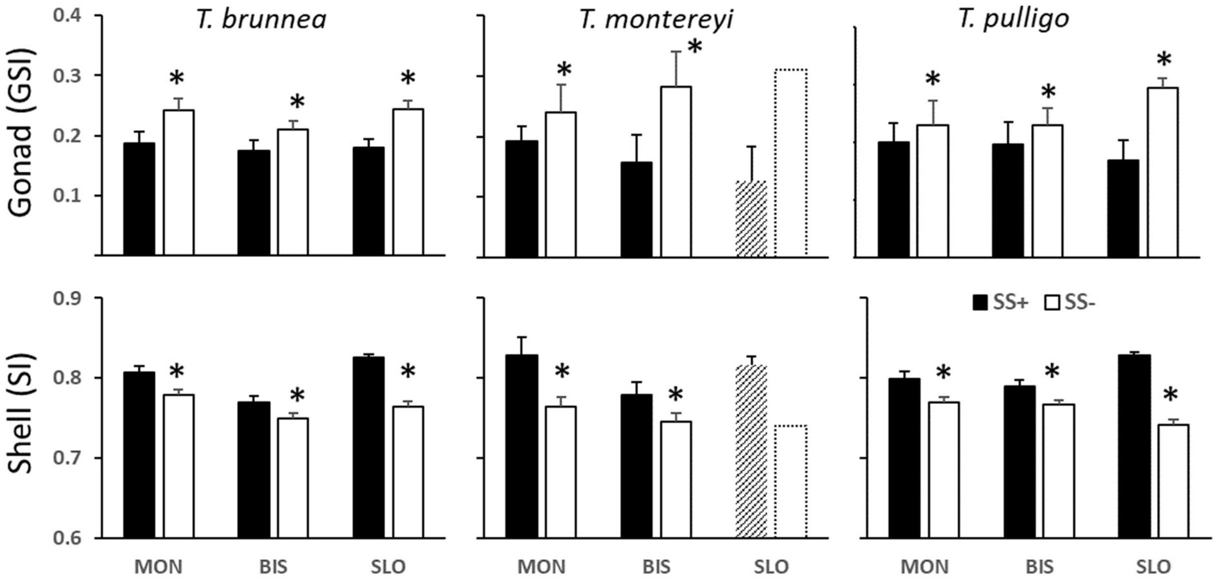

**Figure 3.** Allometric growth investment for *T. brunnea, T. montereyi, and T. pulligo*, based on mean proportional allocation to reproductive tissue (gonadosomatic index [GSI]) and defensive growth (shell index [SI]) at paired predator present (SS+)/ absent (SS-) sites in each of the three study regions. Error bars represent one standard error of the mean and asterisks denote statistically significant differences between paired sites. Sample sizes of *T. montereyi* numbers in SLO were extremely low for both SS+ (n = 3) and SS- (n = 1) field collections, and these were excluded from analyses (denoted by dashed and dotted lines).

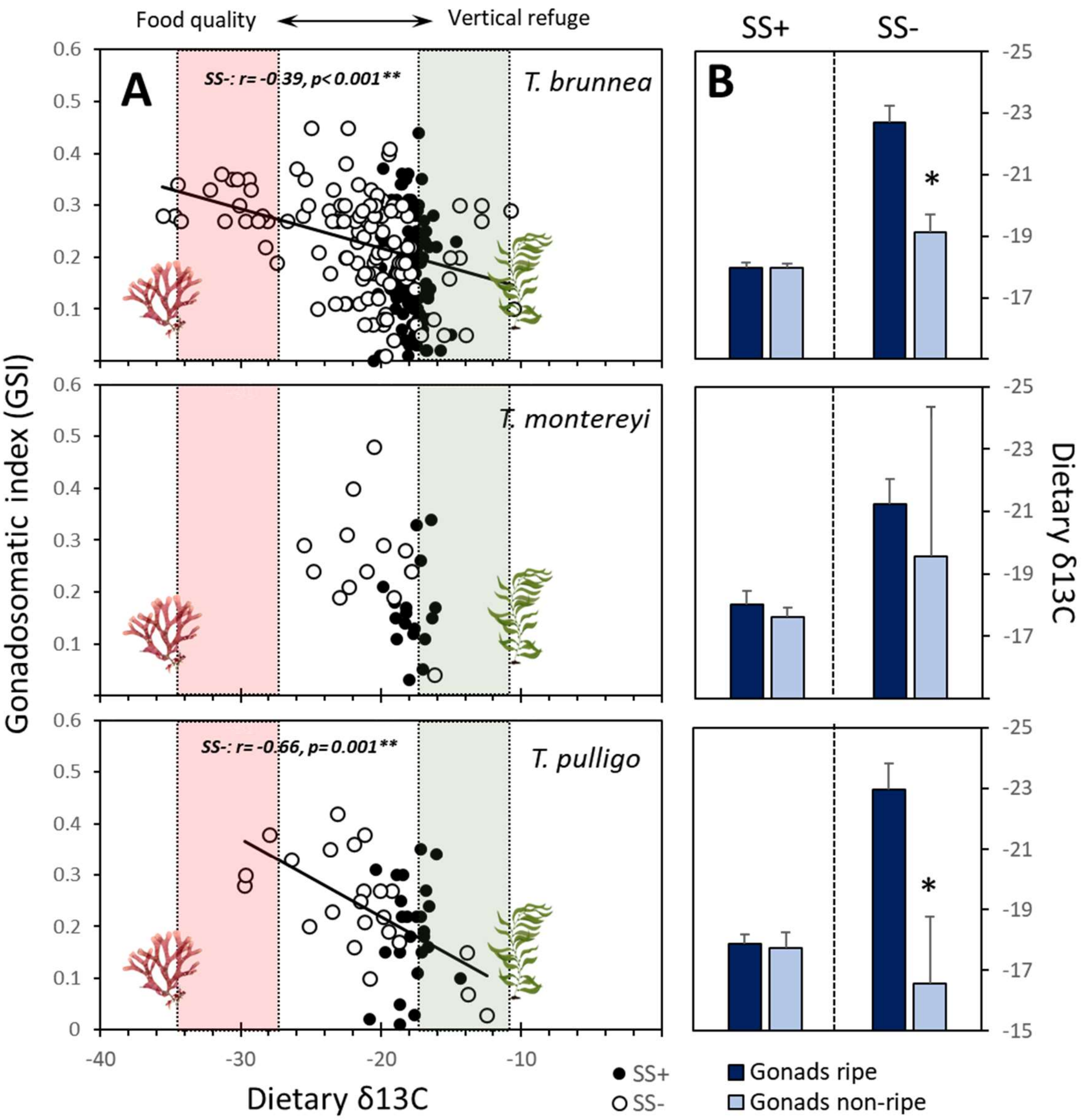

**Figure 4.** (A) Relationship between individual *Tegula* dietary (δ13C) isotopic signatures and snail reproductive investment for *T. brunnea* (n = 221)*, T. montereyi* (n = 31)*,* and *T. pulligo* (n = 48)*,* in the presence (SS+, closed circles) and absence (SS-, open circles) of predatory sea stars, pooled across study regions. Shaded areas represent 95% confidence intervals for isotopic δ13C signatures of *Macrocystis pyrifera* (green color) and benthic red algae (red color) collected across all three regions (n = 60 samples per alga); snail δ13C values were corrected for isotopic fractionation using a trophic enrichment factor (TEF) of 1.5‰ to facilitate a direct comparison with producer values. For each of the three *Tegula* species, bar graphs (B) compare mean (± SE) dietary δ13C signatures for snails displaying ripe vs. non-ripe gonad condition within predator present (SS+) and predator absent (SS-) samples. Asterisks denote significant differences between ripe vs. non-ripe individuals within a given site type (i.e., SS+/SS-).

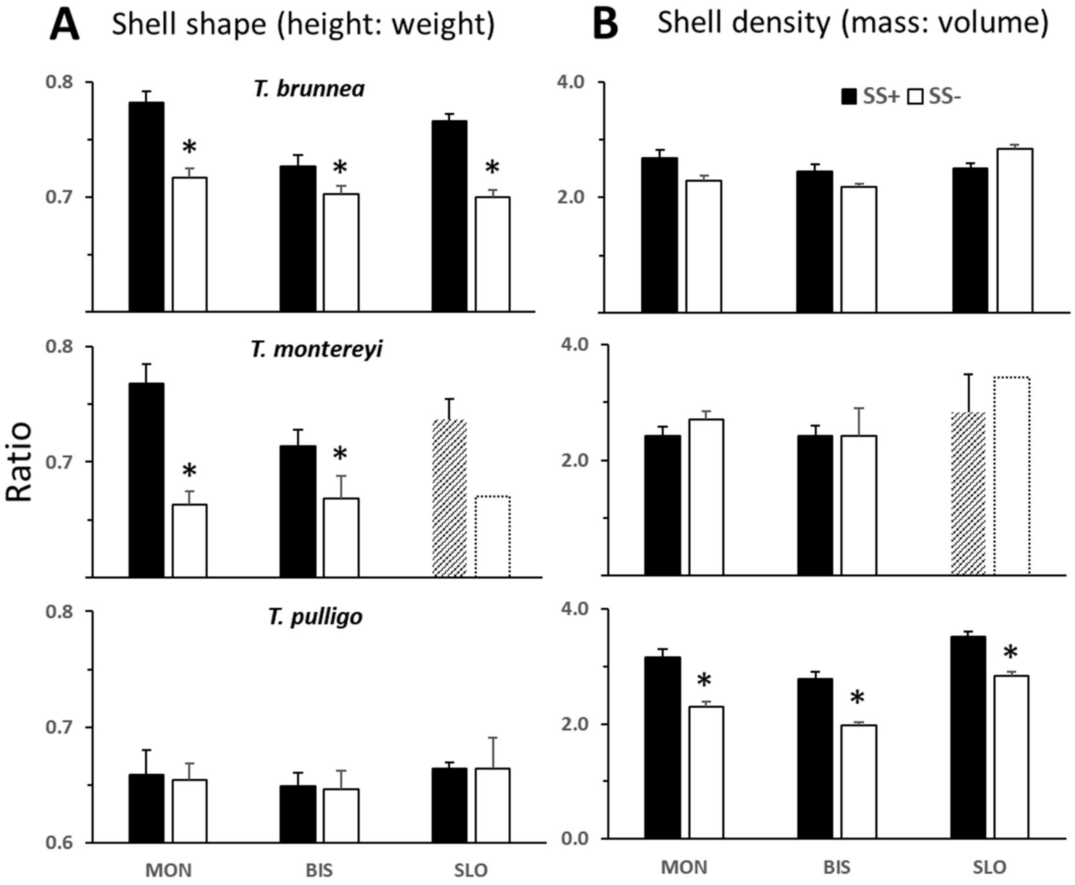

**Figure 5.** (A) Mean shell height/width ratio (SH:SW) and (B) shell density (mass[g]/estimated volume [cm$^3$]; calculated as a proxy for thickness) displayed by each *Tegula* species across paired predator present (SS+) and absent (SS-) sites in each of the three study regions. Error bars represent one standard error of the mean and asterisks denote statistically significant differences between paired sites within regions. Sample sizes of *T. montereyi* numbers in SLO were extremely low for both SS+ (n = 3) and SS- (n = 1) field collections, and thus, were excluded from analyses (denoted by dashed and dotted lines).

# CHAPTER 3

# Modeling potential ecosystem-level impacts of a behaviorally mediated trophic cascade driven by sea star wasting disease in California kelp forests

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## ABSTRACT

Non-consumptive, behaviorally-mediated predator-grazer interactions been widely proposed as a critical component of top-down regulation in subtidal food webs, but have rarely been demonstrated at an ecologically relevant scale in the field. Here, we utilize the recent outbreak of sea star wasting disease along the California coast as a natural experimental system in which to evaluate the effects of predator release on the population dynamics and grazing impacts of the herbivorous turban snail *Tegula* spp., a highly abundant macroalgal consumer in regional kelp forest ecosystems. Preliminary model results indicate that even low levels of predator presence have the potential to exert strong, cascading regulatory effects on grazing activity that are reflected in increased abundance and stability at lower trophic levels (i.e., giant kelp, benthic understory algae). Conversely, on reefs where *Tegula* populations have been released from sea star regulation, food web dynamics are highly unstable and overutilization of slow-growing benthic algal groups results in population crash in nearly all model scenarios. Interestingly, population responses in these models are strongly non-linear, and at high levels of kelp production (i.e., intrinsic growth rates corresponding to ideal environmental conditions), predator removal has an opposite, stabilizing effect, even though macroalgal production converges at lower levels compared to predator present models . Here, we present evidence that significant behaviorally-mediated predator-grazer regulation continues to occur even at extremely low sea star densities that would be considered negligible in traditional direct consumption predator-prey models. If this is the case, it is likely that the role of predator presence in maintaining ecosystem stability has been greatly underestimated in kelp forest food webs. Additionally, because the net impacts of grazer release appear to be highly dependent on local kelp production (and thus, environmental conditions), understanding and managing the predatory species responsible for

maintaining trait-mediated grazer regulation may be critical to conserving ecosystem function in coastal food webs increasingly stressed by climate change and other anthropogenic impacts.

***Key words:*** *Trophic cascade, TMII, prey-switching, sea star wasting disease, predator-prey model, top-down regulation, kelp forests*

## INTRODUCTION

Understanding the effects of predator-induced "top-down" grazing regulation is critical to effective management of subtidal rocky reefs along the California coast, where macroalgae such as the giant kelp, *Macrocystis pyrifera,* are essential to ecosystem function (Falkenberg et al. 2012). Previous studies suggest that predator removal has the potential to drive kelp forest decline by disrupting grazing regulation of benthic herbivores (e.g., Hughes et al. 2013). However, existing research in California has focused almost exclusively on urchins, even though recent trophic interaction models identify mid-sized gastropods (e.g., *Tegula spp.)* as a critical and overlooked source of macroalgal consumption whose relative impact will likely increase as fishery-targeted grazers (i.e., urchins, larger invertebrates) are removed from the ecosystem (Sala and Graham 2002). Macroalgal preferences of *Tegula* snails appear to be driven by the presence of benthic predators (primarily sea stars of the genus *Pisaster* and *Pycnopodia*; Watanabe 1984a,1984b). Thus, evaluating the extent of top-down regulation for this grazer group could provide critical insight to the processes influencing macroalgal cover off the California coast, which are insufficiently explained by urchin-only models (e.g., Guenther et al. 2012). This is particularly true for coastal kelp forests in central and northern California, where urchins are generally present only in low densities, and *Tegula* represent the dominant macroalgal grazers. The sea star-*Tegula* snail food chain is relatively simple, making it an ideal candidate for a trophic cascade model. Sea stars account for over 90% of snail mortality, and snails utilize two major food groups: giant kelp, which provides excellent protection from sea stars but low nutritional content, and understory red/brown macroalgae, which offers high nutritional value but little protection from predators (Figure 1). Sea stars can reduce *Tegula* populations directly through consumption, but also induce complex behavioral and life history modifications in snails that have strong potential effects on population growth. When sea stars are present, snails spend a larger proportion of time on kelp (reducing nutrient intake from benthic algal groups) and also spend a greater amount of time hiding in their shells rather than foraging. Additionally, snails on reefs where sea stars are present typically allocate more energy to defensive growth rather than somatic growth or reproduction, displaying thicker shells and small gonads than snails at reefs with no sea stars.

The recent outbreak of sea star wasting disease (SSWD) in California represents a unique opportunity to conduct a natural experiment quantifying predator-*Tegula* grazer regulation on an ecologically relevant, reef-wide scale that is not otherwise achievable with conventional manipulative studies. Here, we utilized a paired experimental design (six sets of disease [+,-] sites from Bodega Bay – Santa Barbara; Figure 1) in which we compared *Tegula* macroalgal association, dietary preference, and life history characteristics at disease-free reefs where sea stars are still present vs. reefs where sea stars have been completely extirpated by SSWD. Based on empirical data from 2015-16 field surveys and *Tegula* collections, combined with published life history parameters and physiological requirements of study organisms (or ecologically similar species), we have developed a preliminary trophic interaction model evaluating potential effects of sea star predation release on population dynamics of *Tegula*, giant kelp (*Macrocystis pyrifera*) and benthic understory algae (primarily *Rhodophyta, Dictyoneuropis*).

Unlike a majority of quantitative trophic cascade approaches which have focused largely on direct effects of grazer consumption (see Heath et al. 2014 and citations therein), the model presented here is based solely on behaviorally mediated trophic responses and morphological adaptations of *Tegula* snails to the presence/absence of sea star predators (Figure 2). Annual mortality in this early version of the model is held constant at 0.27, based on published *Tegula* survivorship curves (Sharp 1969). The reasoning behind this is twofold. First, observed densities of predatory ochre stars (*Pisaster*) at all study sites were consistently low ( ≈ 0.01 – 0.1 per square meter, well below historical levels), and larger sunflower stars (*Pycnopodia*) were completely absent. Annual *Tegula* consumption rates for *Pisaster* are estimated on the order of 20-30 snails per sea star (Watanabe 1984a), and given that snail densities at many locations were well over 10 individuals/m$^2$, it is unlikely that direct predator-induced mortality is exerting a significant impact on snail population dynamics at the current low sea star densities. Furthermore, an increasing number of studies suggest that non-consumptive effects of predators may have greater impacts on herbivore grazing than direct consumption (Krivan and Schmitz 2004; Reynolds and Bruno 2013). Thus, while linear (e.g., Lotka-Volterra) and density-dependent non-linear models of prey consumption rates (e.g., Holling, 1973) have been reasonably well explored, models incorporating behavioral responses of prey and subsequent alterations in prey fitness and reproduction have rarely been examined.

## METHODS

### *Field collections and surveys*

Three sets of paired sea star (SS+/SS-) sites were sampled between August 2015 and January 2016. Paired sites were located within 8 km of each other and specifically chosen to be comparable in habitat quality, topography, slope face, and wave exposure. At each site, visual surveys were conducted to determine sea star and *Tegula* density/distribution, giant kelp abundance, and understory algal coverage. Counts of sea stars (by species and size class) and kelp sporophytes (including frond counts per individual) were conducted along six 30m x 2m tape transects at each site, and understory algal abundance (percent cover by genus) was quantified based on image analysis of benthic quadrat photographs taken at 5m intervals along each tape transect. Vertical *Tegula* counts were conducted on two randomly selected mature kelp sporophytes on each transect, and benthic *Tegula* counts were conducted within the quadrats used for benthic photographs. Vertical snail counts on kelp were then extrapolated to obtain density estimates based on transect kelp sporophyte densities.

At each site, 60 adult *Tegula brunnea* were collected for morphometric measurements and dietary stable isotope analysis (which is not included here but will eventually be used in place of visual data to inform dietary proportions in models). Based on shell ring counts and published age distributions, all *Tegula* selected for analysis were between 1-2 years of age (approximately 20-24 mm diameter). This size was targeted partially because it represented the majority of individuals observed at each location, and partially because it ensured that all specimens were reproductively mature (Watanabe 1984a) and had settled on adult habitat after the initial outbreak of sea star wasting disease in 2013. This allowed us to examine potential differences in allometric growth patterns, which would be established shortly after settlement in response to local predator conditions (Fawcett 1984). For each individual, gonadosomatic index (GSI) was calculated as:

$$\frac{\boldsymbol{Gonad\ Dry\ Weight}}{\boldsymbol{Total\ Tissue\ Dry\ Weight}}\ \boldsymbol{x\ 100}$$

following the methods of Watanabe (1984b). This standard measure is widely utilized for comparing reproductive potential across multiple populations, and here, empirical GSI estimates allowed us to calculate specific intrinsic annual growth rates ($r_S$) for sea star (+/-) populations (Appendix A).

### ***Modeling approach***

Based on initial survey data from four SS+ sites with highly variable sea star counts (min: 1-2 predators per transect [Big Sur], max: 10-15 predators per transect [Monterey]), we determined that the behavioral response of *Tegula* to sea star presence appears to be largely independent of predator density. All four SS+ snail populations (including Big Sur) consistently displayed > 90% utilization of vertical habitat on giant kelp, while *Tegula* at corresponding SS- sites displayed relatively even distribution between giant kelp (40-60%) and benthic algal groups. Reproductive allocation (GSI) was also consistent across SS+ sites (mean = 0.16. SD = 0.02) and significantly lower than mean GSI at SS- locations (mean = 0.22, SD = 0.01). This greatly simplified our modeling approach because it allowed us to treat sea star presence as a bimodal condition rather than a continuous variable. Thus, we created two separate SS+ and SS- models evaluating only snail population growth (dS/dt), kelp population growth (dM/dt) and benthic algal growth (dA/dt; Table 1 and Appendices A-C).

For simplicity, transect densities for all three populations were converted into biomass densities (g dry weight/m$^2$) using published, species-specific age-growth curves (for *Tegula;* Lord and Shanks 2012), frond count-biomass conversions (for giant kelp; Reed et al. 2008), and percent cover-biomass conversions (for benthic understory algae; Harrer et al. 2013). For the remainder of the text, all references to algal/snail abundance or growth are referring to biomass density estimates rather than discrete counts of individuals.

Modeling framework for both algal groups (giant kelp [M] and benthic algae [A]) was based conceptually on the following fixed-predation, single consumer, dual-resource model:

$$\begin{aligned}
\frac{dR_1}{dt} &= r_1R_1(1-\tfrac{R_1}{K_1}) - \Lambda_{C_1}R_1u_1C \\
\frac{dR_2}{dt} &= r_2R_2(1-\tfrac{R_2}{K_2}) - \Lambda_{C_2}R_2u_2C \\
\frac{dC}{dt} &= u_1C(e_1\Lambda_{C_1}R_1 - m_1 - \Lambda_{P_1}P) \\
&\quad + u_2C(e_2\Lambda_{C_2}R_2 - m_2 - \Lambda_{P_2}P)
\end{aligned}$$

described in Krivan and Schmitz (2004), with several important modifications. This modeling framework was initially chosen because it incorporates both grazer preference (u) and relative abundance of each resource group (R1, R2). In our model, the preference term (u) is replaced by p (proportion of time spent on giant kelp) and 1-p (proportion of time spent on benthic macroalgae; Table 2). We also modified the giant kelp equation to incorporate a term for annual biomass loss (*l*) due to non-grazing erosion and dislodgement, which has been estimated at approximately 0.13 for temperate kelp forests (Bettignies et al. 2015). For both kelp and benthic algae, carrying capacity ($K_M$, $K_A$) in our model is multiplied by a habitat term (h), which represents the proportion of benthic surface consisting of hard bottom habitat at a given location. For all model runs presented here, h was held at 1, representing continuous reef. However, for patchy reef configurations with large areas of sand or sediment unsuitable for algal attachment, adjusting h may be necessary to avoid overestimating carrying capacity. Finally, the terms used to incorporate relative resource abundance were modified from the original model (where grazing is directly proportional to R1 and R2) into abundance ratios (M/(A+M) for dM/dt, A/M for dA/dt). This modification was performed because the original model was designed to describe the behavior of mobile grazers moving from patch to patch, for which R1 and R2 are reasonable estimates of encounter rate. In our system, the consumer *Tegula* spends 100% of time on either kelp or benthic algae, and thus, a proportional approach to resource abundance is more appropriate than direct rate of encounter.

The modeling framework for snail population dynamics presented here is modified from a traditional logistic population growth model, which is substantially different from the modified Lotka-Volterra approach taken by Krivan and Schmitz and a number of other trophic interaction models, including the majority of those commonly applied in urchin-kelp grazing systems (e.g., Mohn and Miller 1987). Although urchins and herbivorous snails fill similar ecological niches in kelp forest food webs, marked physiological differences between the two groups suggest that models developed for urchins (and other echinoderms) are insufficient to describe gastropod population dynamics and trophic interactions (Andrew 1989). Specifically, herbivorous snails have more complex musculature, higher investment in soft tissue, and significantly higher respiration rates than urchins, which have relatively simple body plans and can remain dormant for months or longer without feeding. Consequently, it is hypothesized that gastropods are more likely than other grazers to exhibit density-dependent population dynamics (Andrew 1989), and this is supported by a number of empirical studies reporting decreased growth, increased mortality, and reduced reproduction in marine snails under conditions of food limitation or high population density (e.g., Watanabe 1984b, Bosman and Hockey 1988, Wilson et al. 1999, Schmitt 1996). In our model, carrying capacity is not a constant value but rather a function of food availability (kCal/m$^2$), which is calculated from kelp and algal abundance using the following formula:

$$\boldsymbol{C(A,M) = dA + bM}$$

where d and b are constants representing published caloric values for kelp and benthic algae (kCal/g) in Watanabe (1984b). Thus, the function used to represent calorie density (per square meter) in snail population models is as follows:

$$\boldsymbol{C(A, M) = 2.87A + 2.26M}$$

All models used in simulations for both SS+ and SS- populations are outlined in Table 1, with an explanation of model variables and parameters in Table 2. More detailed explanations of model development and parameter calculations are outlined in Appendices A-C.

### *Model simulations*

Analysis of differential growth equations was conducted in XPPAUT (Ermentrout 2002) using a 4th order Runge-Kutta integration method with adaptive steps (Tolerance : 0.001, Min Step : 1e-12, Max Step : 1, Total time: 1000). In order to evaluate potential effects of algal community structure on system stability, all model simulations were run for the following three sets of initial conditions, which were selected based on three distinct algal community types observed in field surveys:

| | | |
|---|---|---|
| ***Moderate kelp cover, moderate understory algae:*** | ***M(0) = 150*** | ***A(0) = 70*** |
| ***Dense kelp cover, sparse understory algae:*** | ***M(0) = 250*** | ***A(0) = 30*** |
| ***Sparse kelp cover, dense understory algae:*** | ***M(0) = 50*** | ***A(0) = 100*** |

On reefs with moderate kelp coverage and canopy density (representative of average conditions observed on transects), giant kelp and benthic understory algae can coexist at relatively high abundance. However, intrinsic growth rates of red and brown understory algae are largely dependent on light penetration (irradiance; Raikar et al. 2001), so on reefs with dense kelp canopy cover, benthic algal growth is typically low due to shading effects. Conversely, reefs with sparse kelp are often characterized by abundant understory algae, but these systems may also be more vulnerable to destructive grazing, given that growth rates of benthic algal groups are commonly 1-2 orders of magnitude lower than giant kelp (Reed et al. 2008; Cavanaugh et al. 2013; Nejrup et al. 2013).

Each of the three algal community types was evaluated for a series of initial snail biomass values S(0) = [1, 5, 10, 15, 20, 25, 30, 35, 40] to determine the range of grazer population densities over which these ecosystems are likely stable. Additionally, over the range of initial snail population densities where transitions from stability to instability were observed, models were repeatedly run with stepwise increases in initial snail density to characterize dynamics surrounding transition points.

Finally, because the resiliency of kelp forest ecosystems to high levels of grazing pressure is thought to be largely dependent on the rapid growth rates and biomass production of the foundation algal species, giant kelp *Macrocystis pyrifera*, models were simulated using three different estimates of intrinsic annual biomass growth for kelp ($R_M$):

***Avg. reported annual growth rate (current conditions):*** $R_M = 8$
***Max. annual growth rate (ideal environmental conditions):*** $R_M = 12$
***Min. annual growth rate (poor environmental conditions):*** $R_M = 4$

Model simulations and results are summarized in Table 3. The low kelp production scenario ($R_M = 4$) is of particular interest in predictive models, given that the conditions under which these growth rates are currently observed for giant kelp (i.e., high sea surface temperature, high water turbidity, eutrophication) are largely representative of environmental conditions predicted under climate change scenarios and increased anthropogenic degradation of coastal habitats (Steneck et al. 2002). Consequently, examining results from this model may provide insight into potential interactions between altered environmental conditions and trophic regulation within coastal food webs.

## RESULTS AND DISCUSSION

For sea star present (SS+) models, the qualitative behavior of grazer (S) and producer (M,A) populations remained relatively consistent for all three macroalgal community types (med, dense, sparse kelp canopy) and across all three kelp productivity scenarios (average RM=8, maximum RM=12, minimum RM = 4; Table 3). For a wide range of initial snail biomass values (S0), both giant kelp and benthic algal groups approached carrying capacity over time (KM=208, KA=122), while snail biomass remained constantly low or approached 0. At some threshold value of S0, each system eventually became unstable, causing all three populations to crash to 0 (Figures 3-5). As would be expected, the maximum kelp production scenario supported the highest initial biomass of grazers before becoming unstable (S0= 32-34 g snail/m2), compared to 23-24 g snail/m2 for the average production model and 13 g snail /m2 for the minimum production model. Additionally, for all three kelp production scenarios, the moderate kelp coverage community type appeared to be slightly more stable (based on higher maximum S0 supported) than either the dense kelp or sparse kelp initial algal conditions (Table 3). These results are consistent with empirical observations from field surveys, where relatively high *Tegula* densities at sites with sea stars present appear to be sustained with little evidence of destructive grazing impacts or population-level effects on macroalgal communities. Resilience to intense grazing pressure is likely maintained in part because sea star presence drives a disproportionate number of snails to utilize giant kelp as a food source, which has a much higher growth rate than benthic algae and is also less nutritious, leading to slower *Tegula* biomass accumulation and lower reproductive output. Additionally, allometric reproductive allocation (GSI) is inherently lower in *Tegula* populations exposed to predators, and this is represented in the model by a lower intrinsic growth rate rS compared to predator-free populations (Appendix A).

In contrast, removal of predatory regulation in the sea star absent (SS-) models appeared to have a destabilizing effect on local food web dynamics, at least in lower productivity systems. For both the average (rM=8) and minimum (rM=4) kelp production scenarios (Figures 3 & 5), system dynamics were unstable for all potential values of initial grazer biomass (S0), with algal and snail populations crashing to 0 even at extremely low levels of grazing pressure (Table 3). Interestingly, in the high kelp production scenario (rM=12), predator removal appeared to have an opposite, stabilizing effect on local food web dynamics, with snails, kelp and benthic algae all converging to non-zero stable states over a relatively wide range of initial grazer conditions (Figure 4, Table 3). The dynamics driving these patterns may be similar to the competitive coexistence-adaptive predator effect proposed by Krivan (2003) and Krivan and Schmitz (2004), where flexible predators utilizing food groups proportional to relative environmental abundance can have a strong stabilizing effects on basal resource populations as well as consumers. Still, even under the high production scenario (rM=12), equilibrium population densities for both giant kelp and benthic red algae are substantially lower under SS- model conditions compared to SS+ models, suggesting a substantial long-term loss of ecosystem productivity following local predatory sea star extinction. Additionally, in the specific model system here, it should be noted that the observed behavioral shift in *Tegula* grazing activity following disease-induced predatory sea star removal switches preferential snail algal consumption from a rapidly growing, highly abundant food source (i.e., giant kelp) to a much slower growing producer group (benthic red understory algae) that naturally occurs at comparatively low biomass density, even in pristine, undisturbed kelp forest habitats with predatory regulation intact.

In classic predator-prey systems, prey-switching between fast- and slow-growing resources based solely on relative abundance/encounter rate is notoriously unstable, particularly at low resource densities as population levels approach zero, because asynchrony in population responses can lead to overutilization and local extinction of the slow growing food source if consumers do not respond perfectly and immediately (i.e., with complete, accurate knowledge) to diminishing environmental abundance (Abrams and Matsuda 2003). However, in healthy kelp forest systems here where predatory sea star regulation remains intact, the disproportionate (0.90) preference for the faster growing food source (i.e., giant kelp) observed during empirical field surveys suggests that the vertical predator avoidance response displayed by *Tegula* in response to benthic predation threat from Pisaster and/or Pycnopodia sea stars may serve as a buffer to prevent unstable oscillations associated typically associated with slow-fast growth resource switching between kelp and benthic red algae. However, when predators experience extensive local extinctions at a reef-wide scale (as with SSWD), this behavioral constraint is removed, resulting in a 50:50 giant kelp: benthic algae preference that essentially returns to free resource switching based on relative abundance, often leading to instability in predator absent (SS-) population models under certain sets of initial conditions (e.g., high initial grazer densities S0, sparse understory algae initials) where population crashes occurred within < 1 year of model initiation due to rapid depletion of benthic red algae at a time-scale too short to be offset by any population level response.

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# TABLES

## *Table 1. Summary of dynamic population models by trophic group*

***Grazer (Tegula spp.)***

*General form:*

$$\frac{dS}{dt} = r_S S\left[1 - \left(\frac{S}{kC(A,M)}\right)\right] - fS$$

*Sea star presence/absence models:*

$$\frac{dS}{dt}_{(SS+)} = 0.264 \cdot S\left[1 - \left(\frac{S}{0.23 \cdot C(A,M)}\right)\right] - 0.27 \cdot S$$

$$\frac{dS}{dt}_{(SS-)} = 0.362 \cdot S\left[1 - \left(\frac{S}{0.23 \cdot C(A,M)}\right)\right] - 0.27 \cdot S$$

$$C(A,M) = 2.87A + 2.26M$$

***Benthic producers (red understory algae)***

*General form:*

$$\frac{dA}{dt} = r_A A\left[1 - \left(\frac{A}{K_A \cdot h}\right)\right] - S(1-p)\Lambda\left(\frac{A}{A+M}\right)$$

*Sea star presence/absence models:*

$$\frac{dA}{dt}_{SS+} = 365\left(0.037\left[1 - \exp\left(\frac{-0.284\boldsymbol{E}}{365 \cdot 0.037}\right)\right] - 0.0016\right) \cdot A\left[1 - \left(\frac{A}{122 \cdot \boldsymbol{h}}\right)\right] - S(0.10)(36.5)\left(\frac{A}{A+M}\right)$$

$$\frac{dA}{dt}_{SS+} = 365\left(0.037\left[1 - \exp\left(\frac{-0.284\boldsymbol{E}}{365 \cdot 0.037}\right)\right] - 0.0016\right) \cdot A\left[1 - \left(\frac{A}{122 \cdot \boldsymbol{h}}\right)\right] - S(0.50)(36.5)\left(\frac{A}{A+M}\right)$$

***Kelp producer (Macrocystis pyrifera)***

*General form:*

$$\frac{dM}{dt} = r_M M\left[1 - \left(\frac{M}{K_M \cdot h}\right)\right] - S(p)\Lambda\left(\frac{M}{A+M}\right) - lM$$

*Sea star presence/absence models:*

$$\frac{dM}{dt}_{SS} = \boldsymbol{r_M} M\left[1 - \left(\frac{M}{308 \cdot h}\right)\right] - S(0.90)(36.5)\left(\frac{M}{A+M}\right) - 0.13M$$

$$\frac{dM}{dt}_{SS} = \boldsymbol{r_M} M\left[1 - \left(\frac{M}{308 \cdot h}\right)\right] - S(0.50)(36.5)\left(\frac{M}{A+M}\right) - 0.13M$$

*Parameter evaluation:* $r_M = [8_{avg}, 12_{max}, 4_{min}]$

***Range of initial conditions examined***

S(0) = [1, 5, 10, 15, 20, 25, 30, 35, 40]     M(0) = [150, 250, 50]     A(0) = [70, 100, 30]

### *Table 2. Summary of model values and parameters (see Appendices A-C for further detail)*

| **Variables** | | **Units** |
|---|---|---|
| ***S*** | Snail (*Tegula*) population biomass density | (g dry |
| ***M*** | Kelp (*Macrocystis)* population biomass density | (g dry |
| ***A*** | Understory algae (*Rhodophyta, Dictyoneuropis*) population biomass density | (g dry |
| ***C(A,M)*** | Total combined nutritional availability from both kelp and understory algae per unit | (kCal/m$^2$) |
| **Parameters** | | |
| **$S_0$** | Initial population biomass density of *Tegula* snails used in models- ranges based on observed values in field surveys | (g dry weight/m$^2$) |
| **$M_0$** | Initial population biomass density of giant kelp (*Macrocystis*) used in models-ranges based on observed sporophyte densities in field surveys, with biomass conversions adapted from Reed et al. (2008) | (g dry weight/m$^2$) |
| **$A_0$** | Initial population biomass density of understory algae used in models, ranges based on observed percent cover in field survey quadrats, with species-specific biomass conversions adapted from Harrer et al. (2013) | (g dry weight/m$^2$) |
| **rS** | Estimated intrinsic annual growth rate for *Tegula* snails- calculated using mean observed gonosomadic index (GSI) from sea star (+/-) sites (*Figure 1*), published gonadal egg densities for the intertidal congener *T. funebralis* (Cooper and Shanks 2011), a 0.5 operational sex ratio (field observations), and published survival probabilities during the larval and post-settlement phase for temperate gastropods (Rossetto et al. 2015) | |
| **rM** | Estimated intrinsic annual biomass growth rate for giant kelp- three values [8,12,4] based on published rates for kelp forests experiencing typical, ideal, and sub-optimal environmental conditions (representative of conditions predicted during climate change) | |
| **rA** | Estimated intrinsic annual biomass growth rate for understory algae- calculated based on published growth rate equations for temperate red algae (Nejrup et al. 2013) and typical irradiance values recorded in central California kelp forests (Burgman and Gerard, 1990) | |
| **b,d** | Caloric content of kelp (b) and understory algae (d) used to calculate nutritional availability (C), based on published bomb calorimetry results in Watanabe (1984b) | (kCal/ g dry weight algae) |
| **k** | Conversion factor for adjusting the carrying capacity of *Tegula* based on available caloric content of kelp and understory algal biomass – calculated based on mass balance equations for the intertidal congener *T. funebralis* in Paine (1971) | (g snail biomass/kCal) |
| **h** | Proportion of benthic surface area consisting of rocky hard bottom substrate suitable for kelp or algal growth – here h = 1 for all models to denote continuous reef, but is easily adjusted for reefs that are patchy or interspersed with sand/sediment | Proportion |
| **(p)/(1-p)** | Preferred proportion of diet represented by kelp (p) and understory algae (1-p) based on field observations at low population densities | Proportion |
| **Λ** | Estimated annual algal consumption per unit snail biomass, based on published grazing rates for *Tegula brunnea* reared in temperatures comparable to environmental conditions at field locations (15-19°C; Yee and Murray 2004) | g dry weight$_{algae}$ / (g dry weight$_{snail}$ · year) |

**Table 3.** Summary of all model simulations and outcomes, where S(0), M(0), and A(0) are reported as biomass density (g dry weight/m$^2$)

| | | **Avg kelp production $R_M$=8** | | **Max kelp production $R_M$=12** | | **Min kelp production $R_M$=4** | |
|---|---|---|---|---|---|---|---|
| | | ***Figure 3*** | | ***Figure 4*** | | ***Figure 5*** | |
| | | ***SS+*** | ***SS-*** | ***SS+*** | ***SS-*** | ***SS+*** | ***SS-*** |
| **Macroalgal community type** | *S(0) Range examined:* | 1,5,10,15,20,25,30 | 1,5,10,15,20,25,30 | 1,5,10,15,20,25,30,40 | 1,5,10,15,20,25,30 | 1,5,10,15,20,25,30 | 1,5,10,15,20 |
| | *S(0) Transition range:* | 20-30 | 25-35 | 30-40 | 25-35 | 10-20 | 10-20 |
| ***Med Kelp***<br>M(0)=150, A(0)=70 | **Max S(0) supported:** | **24** | N/A | **34** | **31** | **13** | N/A |
| ***Dense Kelp***<br>M(0)=250, A(0)=30 | **Max S(0) supported:** | **23** | N/A | **32** | **29** | **13** | N/A |
| ***Sparse Kelp***<br>M(0)=50, A(0)=100 | **Max S(0) supported:** | **23** | N/A | **32** | **28** | **13** | N/A |
| | **Convergence behavior:** | M→308<br>A→122<br>S→0 | M→0<br>A→0<br>S→0 | M→308<br>A→122<br>S→0 | ***M→127.8**<br>***A→80.4**<br>***S→30.4** | M→308<br>A→122<br>S→0 | M→0<br>A→0<br>S→0 |

# FIGURES

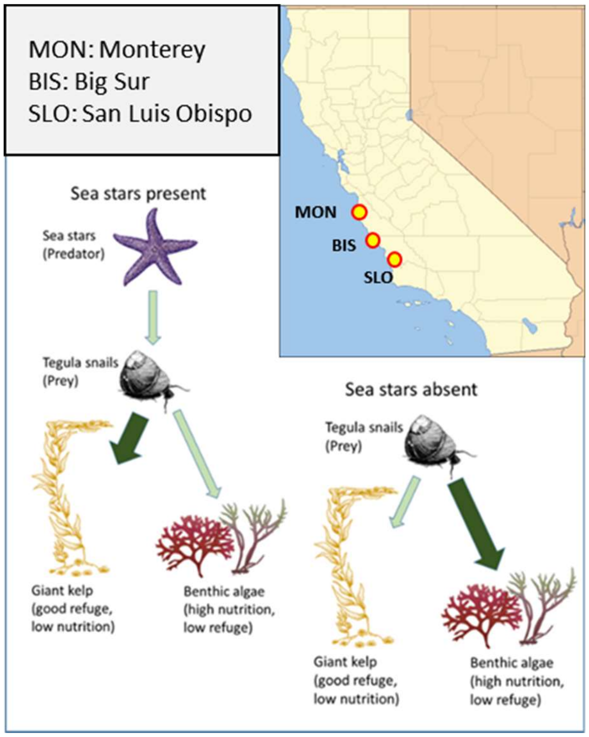

**Figure 1.** Observed grazer-algal relationships for subtidal *Tegula* snails at (SS+) sites where predatory sea stars (*Pisaster spp., Pycnopodia helianthoides*) are present (control, undisturbed scenario) vs. (SS-) sites where these sea star species have undergone complete local extinction following disease outbreak. Shifts in algal grazing behavior described and evaluated here are based on empirical field surveys and dietary analyses between paired (SS+/SS-) sites in each of the three study regions (MON, BIS SLO).

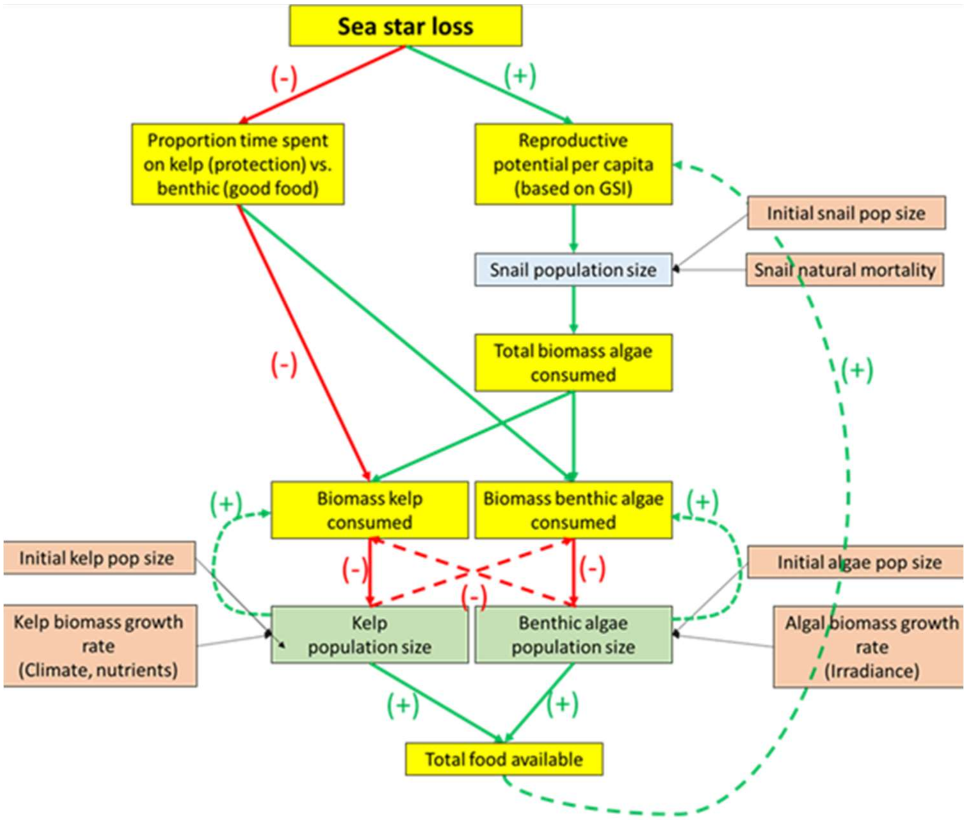

**Figure 2**. Potential *Tegula* behavioral, morphological, and life history responses to disease-driven predatory sea star removal (SS+ → SS-) used to conceptualize model development. Predicted responses of local snail populations to predator release are directly based on several main empirical results from field surveys and sample collections conducted between paired SS+/SS- sites in 2015-2016 (see Chapter 1). These include most notably: (i) increased proportional habitat utilization and consumption of benthic red understory algae (SS+: ≈ 10% → SS- ≈ 45-50%) relative to giant kelp at SS- sites (based on visual field SCUBA transect surveys, tissue stable isotope analysis) and (ii) increased reproductive (gonad) investment (GSI) at SS- sites, likely related to increased benthic foraging access. Solid colored lines denote predicted positive (+, green) and negative (-, red) direct effects of SSWD-driven predatory sea star removal on lower trophic levels, and dashed lines represent potential feedback loops linking algal production to grazer behavior, physiology and/or abundance, including potential prey switching between *Macrocystis* and benthic red understory algae if one food source becomes depleted (see Krivan 2003).

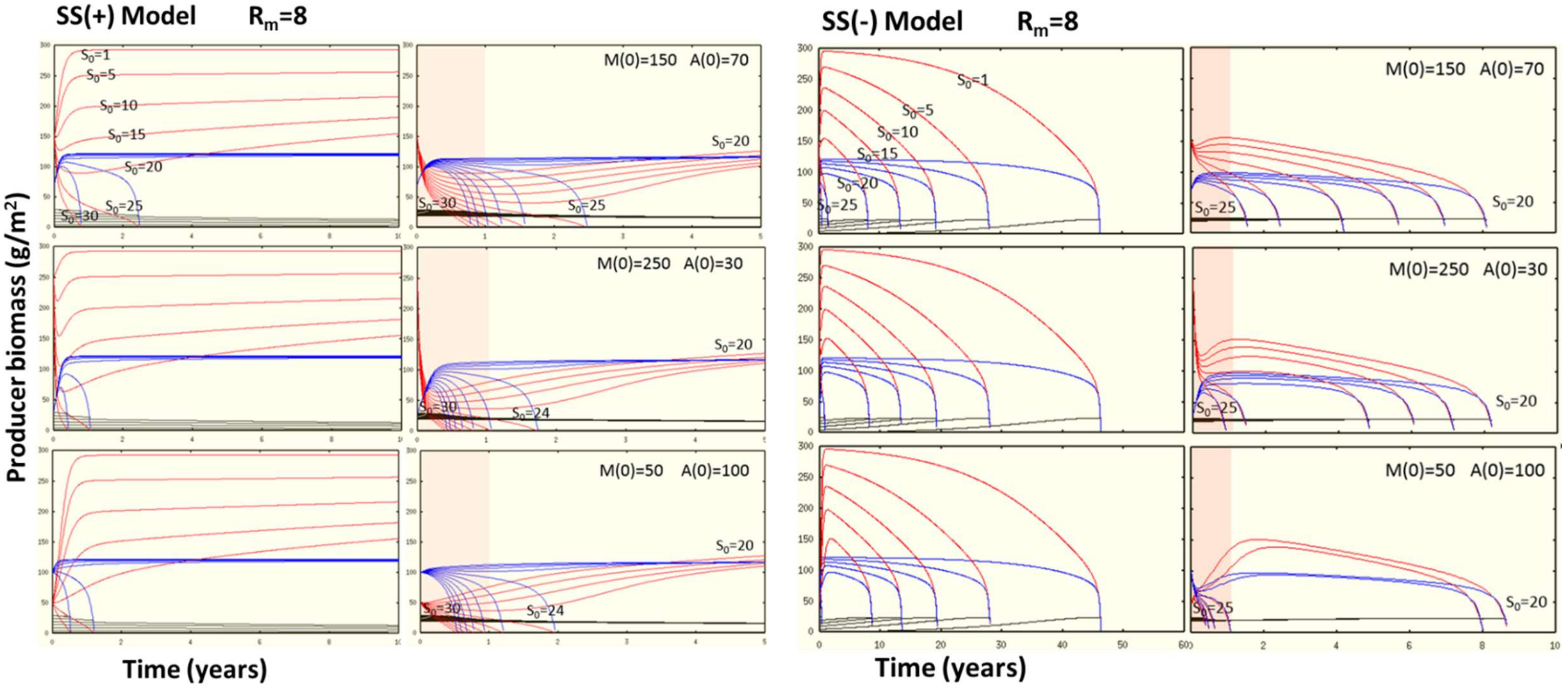

**Figure 3.** Population model results under the average kelp production scenario (Rm = 8) for predatory sea star present (SS+) and absent (SS-) conditions. Each set of models (SS+, SS-) was evaluated over three different sets of initial habitat conditions (representing sparse, medium, and dense kelp, *Macrocystis pyrifera;* see Table 3) and a range of initial *Tegula* snail grazer abundance ($S_0$). Contour lines represent estimated model population biomass densities over time for different $S_0$ values, with *Tegula* (black), giant kelp (red) and benthic red understory algae (blue).

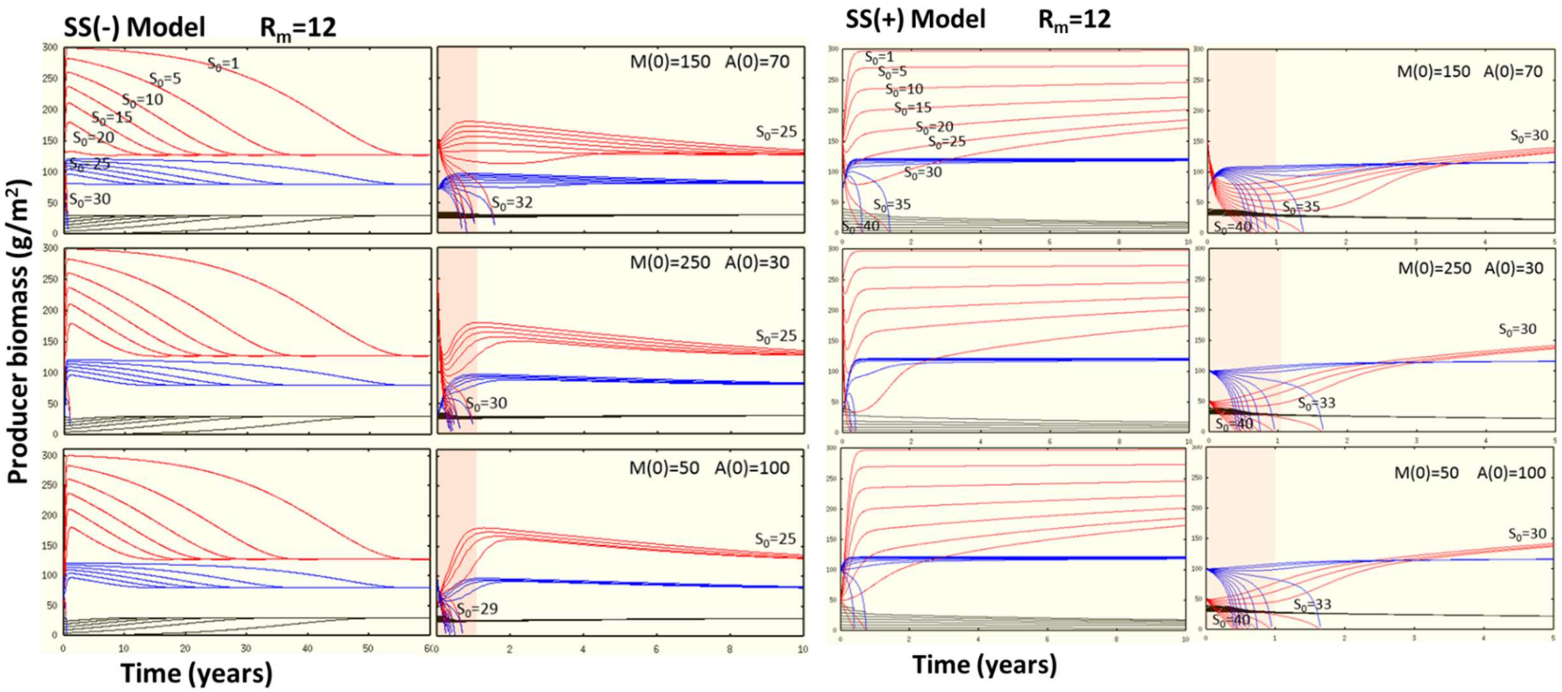

**Figure 4.** Population model results under the maximum kelp production scenario (Rm = 12), representing estimated population outcomes under ideal environmental conditions for predatory sea star present (SS+) and absent (SS-) conditions. Each set of models (SS+, SS-) was evaluated over three different sets of initial habitat conditions (representing sparse, medium, and dense kelp, *Macrocystis pyrifera;* see Table 3) and a range of initial *Tegula* snail grazer abundance ($S_0$). Contour lines represent estimated model population biomass densities over time for different $S_0$ values, with *Tegula* (black), giant kelp (red) and benthic red understory algae (blue).

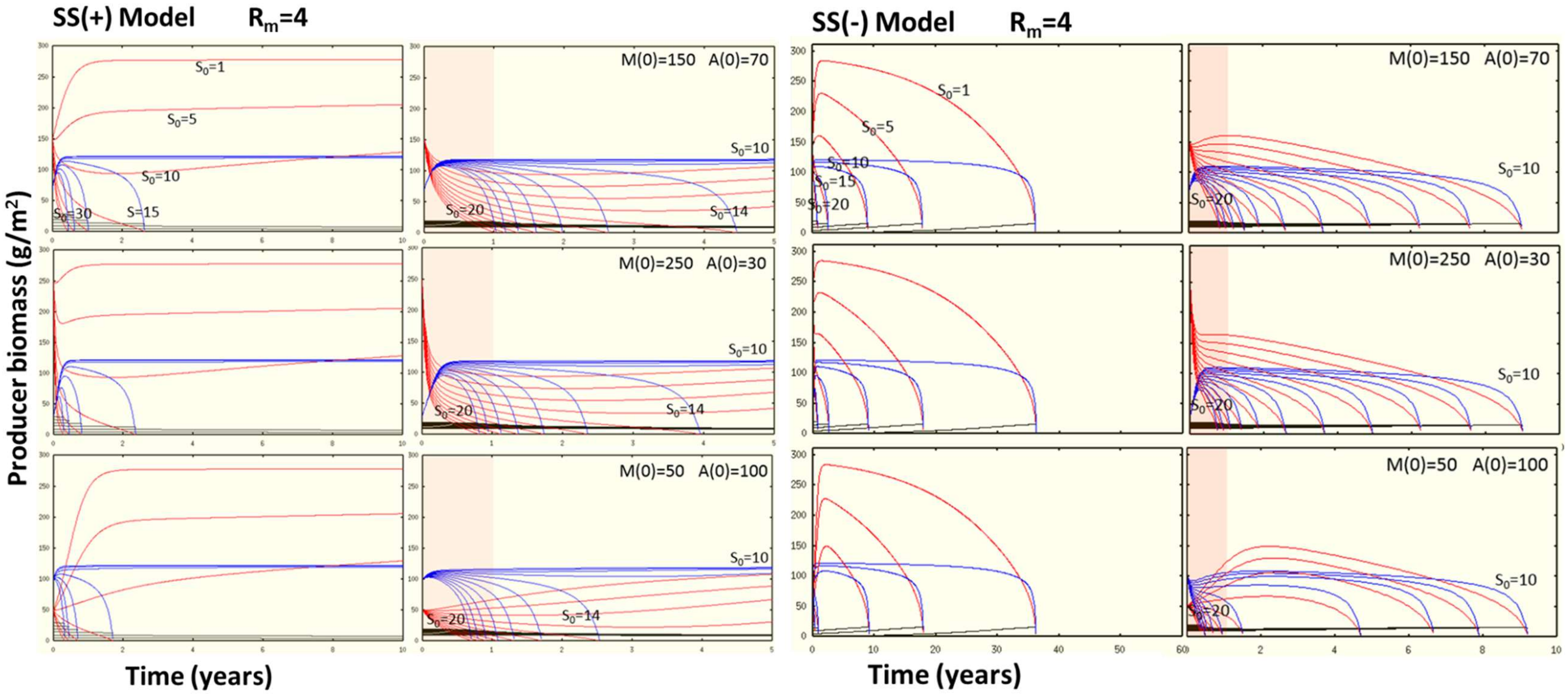

**Figure 5.** Population model results under the low kelp production scenario (Rm = 4) representing suboptimal environmental conditions for algal growth under predatory sea star present (SS+) and absent (SS-) conditions. Each set of models (SS+, SS-) was evaluated over three different sets of initial habitat conditions (representing sparse, medium, and dense kelp, *Macrocystis pyrifera;* see Table 3) and a range of initial *Tegula* snail grazer abundance ($S_0$). Contour lines represent estimated model population biomass densities over time for different $S_0$ values, with *Tegula* (black), giant kelp (red) and benthic red understory algae (blue).

## *Appendix A: Calculation of parameters for snail (Tegula) growth model*

***General model form:***

$$\frac{dS}{dt} = r_S S\left[1 - \left(\frac{S}{kC(A,M)}\right)\right] - fS$$

Calculation of intrinsic population growth rate [$r_S$] using empirically derived GSI values from SS+/- sites, published gonadal egg density for an ecologically similar intertidal congener (Cooper and Shanks 2011), observed 0.5 operational sex ratio from collection specimens, and published probability estimates for survival from larval dispersal to settlement P(survive, settle) and from settlement to year 1 reproductive maturity P(survive, reproduce) in temperate abalone (Rossetti et al. 2015):

$$r_S = GSI \cdot egg\ density \cdot 0.5 \cdot P_{survive,settle.} \cdot P_{survive,reprod.}$$

$$r_S = GSI \cdot (10.983 \cdot 10^4) \cdot 0.5 \cdot (3.09 \cdot 10^{-3}) \cdot (0.01)$$

$$where\ GSI_{(SS+)} = 0.16 \quad and \quad GSI_{(SS+)} = \mathbf{0.22}$$

$$r_{s(SS+)} = \mathbf{0.264}$$

$$r_{s(SS-)} = \mathbf{0.362}$$

Calculation of carrying capacity [$C(A,M)$] based on combined kelp and benthic algae calorie density:

$$C(A,M) = dA + bM$$

$$C(A,M) = 2.87A + 2.26M$$

Calculation of carrying capacity conversion factor from calories kCal to snail biomass:

$k = \frac{kCal\ requirement}{g_{snail}} = 0.23$ (adapted from energy mass budget calculations in Paine (1971))

$f = published\ annual\ mortality\ rate = 0.27$

***Predator presence (SS+/SS-) model forms:***

$$\frac{dS}{dt}_{(SS+)} = \mathbf{0.264 \cdot S\left[1 - \left(\frac{S}{0.23 \cdot C(A,M)}\right)\right] - 0.27 \cdot S}$$

$$\frac{dS}{dt}_{(SS-)} = \mathbf{0.362 \cdot S\left[1 - \left(\frac{S}{0.23 \cdot C(A,M)}\right)\right] - 0.27 \cdot S}$$

$$\mathbf{C(A,M) = 2.87A + 2.26M}$$

## *Appendix B: Calculation of parameters for giant kelp (Macrocystis) population growth model*

### *General model form:*

$$\boldsymbol{\frac{dM}{dt} = r_M M\left[1-\left(\frac{M}{K_M\cdot h}\right)\right] - S(p)\Lambda\left(\frac{M}{A+M}\right) - lM}$$

$\boldsymbol{h} = proportion\ of\ benthos\ consisting\ of\ hard\ bottom\ habitat\ suitable\ for\ kelp\ growth\ (\ reef)$

$\boldsymbol{l} = average\ annual\ population\ loss\ due\ to\ natural\ erosion/dislodgement$ = 0.13

$\boldsymbol{K_M} = carrying\ capacity\ (g\ dry\ weight)\ per\ m^2\ reef$
$= max.\ kelp\ density(indiv/m^2)\cdot avg.\ frond\ count(\#\ fronds/indiv)\cdot depth(m)$
$\cdot frond\ biomass\ conversion(g\ wet\ weight/(frond\cdot m)\cdot dry\ weight\ conversion$

$\boldsymbol{K_M} = (0.2)(20)(10)(110)(0.07) = 308\ (g\ dry\ weight/m^2)$, calculated based on the following:

Transect depth = 10m (field data)

Max. kelp density = 0.2/m$^2$ (max. predicted values in population models; Burgman and Gerard 1990)

Avg. frond count = 20 (empirical counts averaged across field locations [range: 17-24])

Length: biomass conversion = 110 ( [wet weight (g)/ frond length(m)] relationships; Reed et al. 2008)

Dry weight conversion = 0.07 (average water content (93%) for *M.pyrifera* tissue; Watanabe 1984b)

### *Predator presence (SS+/SS-) model forms:*

$$\boldsymbol{\frac{dM}{dt}_{SS+} = r_M M\left[1-\left(\frac{M}{308\cdot h}\right)\right] - S(0.90)(36.5)\left(\frac{M}{A+M}\right) - 0.13M}$$

$$\boldsymbol{\frac{dM}{dt}_{SS-} = r_M M\left[1-\left(\frac{M}{308\cdot h}\right)\right] - S(0.50)(36.5)\left(\frac{M}{A+M}\right) - 0.13M}$$

$\boldsymbol{\Lambda}$ = annual algal biomass consumption per unit snail

$$0.10\frac{g_{algae}}{g_{snail}\cdot d}\cdot\ 365d = 36.5\frac{g_{algae}}{g_{snail}}$$

$\boldsymbol{p} = observed\ preference\ for\ kelp\ with\ predators\ present\ or\ absent$

$[0.9_{SS+},\ 0.5_{SS-}]$

$\boldsymbol{r_M} = mean\ annual\ kelp\ growth\ rate\ under\ under\ ideal, average, and\ poor\ conditions$

$[12_{max},\ 8_{avg}, 4_{min}]$

## *Appendix C: Calculation of parameters for benthic understory algae population growth model*

### *General model form:*

$$\boldsymbol{\frac{dA}{dt} = r_A A\left[1 - \left(\frac{A}{K_A \cdot h}\right)\right] - S(1-p)\Lambda\left(\frac{A}{A+M}\right)}$$

Calculation of annual intrinsic growth rate based on irradiance-growth relationships for temperate red algae (Nejrup et al. 2013)

$$\boldsymbol{r_A} = 365\left(0.037\left[1 - \exp\left(\frac{-0.284E}{365 \cdot 0.037}\right)\right] - 0.0016\right)$$

$\boldsymbol{E} = \left[200_{avg},\ 300_{max}, 100_{min}\right]$, average irradiance

$(\boldsymbol{1 - p}) = [0.1_{SS+},\ 0.5_{SS-}]$

$\boldsymbol{\Lambda} = 36.5\frac{g_{algae}}{g_{snail}}$

$\boldsymbol{K_A} = 122\ g\ dry\ weight/m^2$, carrying capacity

where 122 is the published conversion factor to convert percent cover into biomass (dry weight; Harrer et al. 2008). To estimate carrying capacity, we simply assume 100% benthic coverage so 122 x 1 = 122.

### *Predator presence (SS+/SS-) model form:*

$$\boldsymbol{\frac{dA}{dt}_{SS+} = 365\left(0.037\left[1 - \exp\left(\frac{-0.284E}{365 \cdot 0.037}\right)\right] - 0.0016\right) \cdot A\left[1 - \left(\frac{A}{122 \cdot h}\right)\right] - S(0.10)(36.5)\left(\frac{A}{A+M}\right)}$$

$$\boldsymbol{\frac{dA}{dt}_{SS-} = 365\left(0.037\left[1 - \exp\left(\frac{-0.284E}{365 \cdot 0.037}\right)\right] - 0.0016\right) \cdot A\left[1 - \left(\frac{A}{122 \cdot h}\right)\right] - S(0.50)(36.5)\left(\frac{A}{A+M}\right)}$$