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ECOLOGICAL EFFECTS OF AN INVASIVE MUD SNAIL AND ITS BODY-SNATCHING PARASITES: IMPLICATIONS FOR ORGANIC MATTER CYCLING IN A EUTROPHIC ESTUARY

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Abstract

Rachel Ann Fabian

Ecological impacts of an invasive estuarine mud snail and its body-snatching parasites: implications for he cycling or organic matter in a eutrophied estuary

In this dissertation, I address the implications of a poly-species invasion consisting of a mud snail (*Batillaria attramentaria*) and the novel trematode parasite that uses the snail as a host, and I characterize the effects of this invasion on organic matter cycling in Elkhorn Slough. In Chapter 1, I used field transect surveys and a mark-recapture experiment to show that trematodes induced snails to increase growth and alter their habitat use. In Chapter 2, I used a field enclosure experiment to investigate the effects of parasitic modifications to *Batillaria* on the snail's food resources and on sediment chemistry, showing that parasitic effects on snails have profound effects on benthic community structure and function. In Chapter 3, I investigated *Batillaria*'s interactions with a bloom-forming macroalga. I used a laboratory experiment to show that the snail could facilitate the algae by increasing its growth in nutrient-replete water, and allowing it to persist in low-nutrient water, potentially exacerbating blooms that cause hypoxia and other ecological problems. My dissertation underscores the context-dependency of ecological interactions, and highlights interactions that should be more widely recognized in forward-thinking ecosystem management.

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Introduction

Habitat destruction, fragmentation, and other types of disturbance create conditions that promote species invasions (Marvier et al. 2004). Estuaries are among the most disturbed systems, having been drained and modified for agricultural, industrial, and urban development, and subsequently affected by runoff associated with these uses. Disturbance of estuaries primes these systems for invasion by species that arrive in ballast water, and on ship hulls and aquaculture stocks (Wasson et al. 2005). Species invasions can alter population, community, and ecosystem processes (Ruiz et al. 1997), and they are among the greatest threats to estuarine systems (Bromberg Gedan et al. 2008). My dissertation investigates the ecological effects of an invasive mud snail and its co-invading parasites in a central California estuary that is highly impacted by human activities. Using a combination of field and laboratory work, I examined snails' role in organic matter cycling among constituent pools, and how these effects are mediated by parasites that use the snail as a host.

Elkhorn Slough (~36°45'-36°52' N, ~121°49'-121°42' W) is a 1200 ha tidal wetland complex that opens to the Monterey Bay. Major changes to Elkhorn Slough began after the Gold Rush in the mid-19th century. Tidal control structures were first implemented to permit agricultural land usage and road construction, and the Salinas River mouth was diverted south of the estuary in the early 20th century. These alterations greatly reduced tidal exchange in the slough, as well as freshwater and sediment inputs. The impact of these alterations has been immense; over ⅔ of marshlands within Elkhorn Slough have been degraded or converted to alternate

habitat types (brackish habitats, unvegetated mudflats, agricultural lands; van Dyke and Wasson 2005). The 1948 construction of the Moss Landing Harbor and dredging of an artificial channel increased tidal amplitude and velocity in Elkhorn Slough, making the estuary an erosional system (van Dyke and Wasson 2005). Some areas remain artificially tidally restricted, with decreased tidal range and long water residence times, up to 50 days at some sites in the summer (Hughes 2009).

Nutrient inputs to the slough have increased since the 1970s as more land has shifted toward agricultural use and the human population in the area has increased (Caffrey et al. 2007). The Old Salinas River (OSR) provides the greatest nutrient loading to the main channel (Wankel et al. 2009), and tidal pumping then transports OSR-derived nutrients up the main channel. Carneros Creek and surrounding agricultural lands are also sources of NO_3^- during the wet season (Chapin et al. 2004), but these inputs are generally restricted to areas behind tidal control structures. Monterey Bay also provides marine nutrients, especially during upwelling season (Apr-Oct)(Hughes et al. 2011). Work with dual isotopic signatures ($\delta^{15}N$ and $\delta^{18}O$) indicates that nitrogen that enters the slough is extensively cycled among algal, sediment, and water column pools (Wankel et al. 2009). Although Elkhorn Slough exceeds eutrophication thresholds (established by Bricker et al. 2003) for many criteria, including nutrient concentrations, algal cover and biomass, chlorophyll *a*, hypoxia/anoxia, and sediment quality, it is considered a moderately eutrophied system, ranging from low to hypereutrophic among sites, due to the influence of factors like tidal range (Hughes et al. 2011).

Selection regimes in changing or altered habitats may favor invasive species over natives, and eutrophication and other alterations in Elkhorn Slough, such as artificial hardening of substrata, may shift the competitive advantage to invaders that can tolerate wide ranges of physicochemical conditions. At least 58 invading macro-invertebrates have become established in Elkhorn Slough, and invasive species account for a much greater proportion of invertebrate species in the estuary (11%) than on the adjacent open coast (1%) (Wasson et al. 2005).

Batillaria attramentaria (= *B. cumingi, B. zonalis;* herafter, *Batillaria*), the Japanese mud snail, is a highly successful invader of intertidal habitats in estuaries from Boundary Bay, BC, to Elkhorn Slough, CA. Its native range is from Korea to Japan, where it is often the dominant invertebrate in salt marshes and on mud flats along the northeastern coast of Asia (Miura et al. 2006). *Batillaria* is a deposit feeder, primarily consuming epibenthic diatoms (Whitlatch and Obreski 1980). It is long-lived (~ 6-10 yr), growing to ~40 mm, maturing at ~15 mm at 2-3 years, though growth rates vary greatly among individuals. *Batillaria* is a gonochorous direct developer; females lay eggs in Apr/May that hatch in Jun/July; juveniles grow to 2-3 mm in their first year (Whitlatch 1974, Yamada 1982). *Batillaria* arrived in Elkhorn Slough on Japanese oyster (*Crassostrea gigas*) stocks around 1929, where it has achieved its highest reported densities of > 22,000 individuals per m^2 (Fabian, unpublished data). *Batillaria* displaces its native ecological analog, *Cerithidea californica* (hereafter, *Cerithidea*), where the species have overlapped, including Elkhorn Slough due to superior efficiency of converting food resources to biomass

and lower mortality (Byers and Goldwasser 2001). Being a direct developer without a planktonic stage has limited *Batillaria*'s dispersal, and probably its distribution in the eastern Pacific, but it remains an invasion risk, with two recent invasions of Bodega Bay (2005) and San Francisco Bay (Weiskel et al. 2007).

Batillaria is the first intermediate hosts to trematode parasites that subsequently parasitize a variety of second intermediate hosts (e.g. crabs, fishes), with birds as definitive hosts. Adult trematodes live in the guts of birds, releasing their eggs in bird feces. The snails become infected either by grazing on mud containing trematode eggs or by swimming miracidia that hatch from eggs and seek snail hosts. Inside the snails, the miracidia develop into clones of rediae that inhabit and consume the snails' gonads, leading to permanent castration of snail hosts. The rediae also divide clonally, either producing more rediae or another motile stage, cercariae, that emerge from the snails and swim to second intermediate hosts, in which they encyst as metacercariae. Metacercariae are transmitted to bird hosts when the second intermediate host is consumed; they develop into adults and undergo sexual preproduction inside avian digestive tracts (Figure A) (Sousa 1983, Huspeni and Lafferty 2004, Hechinger 2007). Infection is permanent; snails effectively become extended parasite phenotypes because they suffer reproductive death, thereafter only producing parasites (Hechinger 2010).

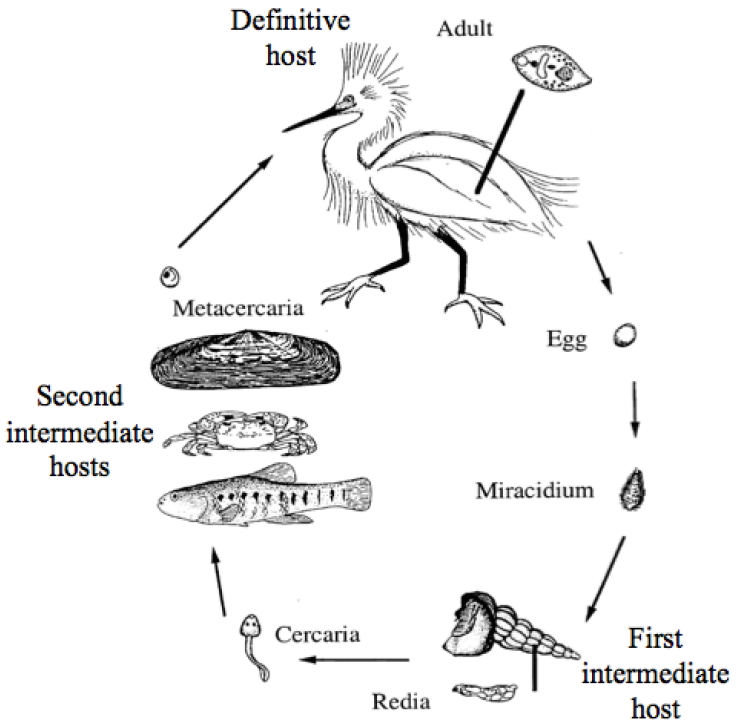

Figure A: Life cycle of trematodes that use *Cerithidea* and *Batillaria* as first intermediate hosts. Modified from Huspeni and Lafferty (2004).

Trematodes are very specific to a single species of first intermediate host, although subsequent life stages often utilize > 1 species, so where *Cerithidea* is replaced by *Batillaria*, the ~18 species of trematode that use the native snail are eliminated and replaced by those that use *Batillaria* (Torchin et al. 2005). In its native range, *Batillaria* is infected by at least 9 morphologically distinct trematode species (Hechinger 2007). Only one species complex (*Cercaria batillariae,* comprised of 8 cryptic species) is present in *Batillaria*'s introduced range. Three cryptic species are present in North America, with 2 being common. *C. batillariae* uses fish as second intermediate hosts (Shimura and Ito 1980, Hechinger 2007), including staghorn

sculpin (*Leptocottus armatus*), longjaw mudsucker (*Gillichthys mirabilis*), and arrow goby (*Clevelandia ios*) in Elkhorn Slough (Torchin et al. 2005). Its avian definitive hosts are not known. *Batillaria* are both abundant and highly parasitized in Elkhorn Slough, with ~46% of snails infected slough-wide (Lin 2006), but infection rates can be as high as 73% at some sites (Chapter 1).

Like many parasite taxa that use multiple hosts, trematodes often alter morphology and behavior of intermediate hosts in ways that facilitate transmission to subsequent hosts (Esch and Fernandez 1994, Sorensen and Minchella 2001), with important ecological effects. Trematodes have been found to cause gigantism in some snail hosts, inducing hosts to continue growth after sexual maturation and to grow at accelerated rates (Baudoin 1975, Sorenson and Minchella 2001; Miura et al. 2006; Hechinger 2010). Host gigantism is probably advantageous for the parasites, since cercarial size and production increase with host shell length (Poulin 2006), and the cessation of snail reproduction should free up energy to fuel somatic growth.

In Chapter 1, I used a series of transect surveys along an intertidal elevation gradient to show that infected snails move to lower elevations on mud flats. Snails are submerged more often at lower elevations, which increases proximity to fishes that the parasites use as subsequent hosts. I also showed that infected snails are larger than uninfected snails. Furthermore, I am the first to show that parasitic effects on host behavior infection are intensified in snails harboring high abundances of actively developing parasites. The prevalence of highly infected snails increased at the lowest elevations occupied by snails. I used multiple regressions to show that snail shell

lengths, prevalence of infection, and prevalence of hosts with high abundances of parasite rediae were inversely correlated to tidal elevation.

The observation that infected snails are larger could be due to parasites choosing larger snails to infect, or the longer exposure time to parasites of larger, older snails. To confirm that trematodes indeed make *Batillaria* grow at faster rates, I used a mark-recapture experiment. I collected snails from the mud flat, and marked their shell apertures with enamel paint. I returned the snails to the mud flat, and collected them 8 weeks later. I used photography to measure the angular growth of the snails, and then dissected the snails to record infection status, and analyzed snail growth rates using ANOVA. These results corroborated my hypothesis that *Batillaria* that are infected by trematodes in Elkhorn Slough grow at faster rates than their uninfected counterparts.

In Chapter 2, I used a field enclosure experiment to investigate the effects of parasitic modifications to the physiology and habitat use of *Batillaria* on its food resources, benthic diatoms, and on sediment chemistry. I used two different size classes of snails as a proxy for snail infection status in the field enclosures, as well as control cages with no snails. I deployed field enclosures at high and low tidal elevations to represent parasite-induced alterations to *Batillaria*'s habitat use. We analyzed the experimental data using a Generalized Linear Model, a Generalized Estimating Equation, and MANOVAs. These analyses showed that infected snails had large effects on diatom size distributions and chlorophyll *a*, an indicator of benthic algal biomass. Moreover, effects of infected snails were opposite at high and

low elevations, with infected snails doubling chlorophyll *a* in high enclosures, and reducing it by 75% in low enclosures. These results emphasize the importance of considering parasitic modifications to hosts in food web modeling and ecosystem-based management.

In Chapter 3, I turned my attention to investigating *Batillaria*'s impacts on *Ulva,* a bloom-forming macroalga, mats of which seasonally accumulate on intertidal mud flats, providing perhaps the most visible indicator of nutrient loading to Elkhorn Slough. Opportunistic algal taxa like *Ulva* respond quickly to nutrient pulses, allowing them to outcompete slower-growing algae, but they decline rapidly when nutrients become scarce. The growth and/or persistence of *Ulva* blooms can be reduced by herbivory, but grazers can also enhance the alga by fertilizing it or stimulating its growth. I used a laboratory experiment to investigate whether *Batillaria* could affect the growth or health (nitrogen content) of *Ulva*. I employed two snail treatments to compare the effects of snail fertilization alone, and the combined effects of fertilization and grazing, as well as control treatments with no snails, and I crossed snail treatments with two nutrient treatments to assess whether nutrient availability affected snails' effects on the algae. Using factorial and one-way ANOVAs, I showed that snails fertilized the algae. In high nutrient treatments, snails increased algal biomass, and in low nutrient treatments, snails mitigated declines to algal health. My results indicate that snails might facilitate the growth of algal mats when nutrients are high, and increase mat persistence when nutrients become limiting, exacerbating algal blooms in Elkhorn Slough.

Macrofaunal grazers, particularly snails, have long been known to play important roles in shaping community structure (e.g. Lubchenco 1980, Silliman and Zieman 2001). My dissertation work demonstrates the importance of considering the ecological implications of parasitic effects to dominant grazer hosts. Trematodes alter *Batillaria*'s growth and habitat use; these effects have profound effects on snail impacts on benthic community structure and ecosystem functions. My work also demonstrates that *Batillaria* can fertilize macroalgae, potentially enhancing the growth and persistence of opportunistic algal mats that afflict coastal and estuarine habitats. Overall, my dissertation underscores the context-dependency of ecological interactions, and highlights interactions that should be more widely recognized. Since anthropogenic pressures to coastal systems, like eutrophication, are expected to continue relatively unabated, and parasitic productivity and host interactions will likely increase with unfolding global change, integration of these processes is essential to forward-thinking ecosystem-based management planning.

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Chapter 1: Effects of parasitic infection on behavior and morphology of an invasive estuarine snail: implications for ecosystem effects in a eutrophic estuary

1.1 Abstract

Parasites can influence ecosystem processes and food web structure both directly and indirectly through their effects on their hosts. Trematodes (flukes) are a diverse group of parasites that use multiple host species to complete their life cycles, and they commonly have adaptations that impact the morphology and behavior of their hosts in ways that facilitate their transmission from one host to the next. This study assesses the effects of a trematode, *Cercaria batillariae*, on its snail host, *Batillaria attramentaria*, in a central California estuary, where the snail and parasite were accidentally introduced on oyster stocks from Japan. Previous work in *Batillaria*'s native range has shown that snails infected with *C. batillariae* grow more than uninfected snails, and move to lower intertidal elevations to increase transmission to subsequent fish hosts. We tested the hypotheses that C. *batillariae* has similar effects on *Batillaria*'s growth and distribution in the introduced range, using a mark-recapture growth experiment and transect surveys. Infected snails grew more, and occupied lower tidal elevations, than uninfected snails, and the abundance of developing parasites within snails was a significant predictor of infected snails' positions on the intertidal mud flat. Our results confirm that *C. batillariae* influences *Batillaria*'s growth and behavior in the species' introduced range, but we also found differences between the host-parasite interactions in the species' native and

introduced ranges. Trematodes are widespread and their abundances are expected to increase with rising global temperatures, so consideration of their effects on hosts, and the spatiotemporal variability of these effects, is critical to sound ecosystem-based management.

1.2 Introduction

Parasites are pervasive in natural systems; ~40% of known species are parasites of the other 60%, and helminths that parasitize vertebrates outnumber their host species by at least 50% (Dobson et al. 2008). Parasites can profoundly influence ecological communities and food webs directly through predatory interaction with host species, and indirectly by modifying host physiology and behavior in ways that affect competition, energy flow, and biodiversity in ecosystems (Minchella and Scott 1991, Hudson et al. 2006). Through these direct and trait-mediated indirect interactions, parasites add many linkages to food webs (Lafferty et al. 2006), sometimes with enough strength to make parasites the keystone species within a system (Sato et al. 2012). Because parasite transmission rates and productivity are expected to increase with increasing global temperatures (Poulin 2006, Marcogliese 2008), an understanding of parasites' ecological effects is essential for forward-looking ecosystem management. Inclusion of parasites in food web accounting and management decisions may be particularly important in aquatic systems, where parasites have documented effects on ecological processes.

Parasites in ecosystems

Parasites that infect multiple hosts often alter the morphology and behavior of intermediate hosts in ways that facilitate transmission to subsequent hosts. Parasites may also alter host resource use (reviewed in Labaude et al. 2015), as well as

strengthen existing predator-prey interactions through changes to host behavior (Lafferty and Morris 1996) or morphology (Bakker et al. 1997). Changing host behavior can create or alter food web links dramatically, leading predators to switch to different prey (Sato et al. 2011), and producing cascading effects through a system (Sato et al. 2012). Because the magnitude of parasitic effects on energy flow is determined by the biomass and energy flow altered by these effects (Kuris et al. 2008), parasitic changes that alter grazing by abundant consumers may exert important top-down controls in food webs that should be incorporated into food web models, especially in eutrophic systems.

Trematode parasites

Trematodes (flukes), a diverse group of parasites that usually use two or more hosts to complete their life cycles are widespread, and they are well studied in freshwater and coastal marine systems. Molluscs are typically the first intermediate hosts, with various invertebrate and vertebrate species serving as downstream intermediate and definitive (final) hosts. Most adult trematodes live in the guts of definitive hosts (birds or mammals), releasing their eggs in feces. Molluscs (usually snails) become infected either by grazing on mud containing eggs, or by swimming miracidia that hatch from eggs and penetrate snail hosts. Inside snails, the miracidia develop into sporocysts and rediae that usually inhabit and consume the snails' gonads, causing permanent castration. These forms divide clonally, and cercariae, an infective free-living form, often develop within active rediae. Cercariae emerge from

snails and penetrate second intermediate hosts (often crustaceans, molluscs, or fish) in which they encyst as metacercariae. Metacercariae are transmitted to definitive hosts when the second intermediate host is consumed; they develop into adults and undergo sexual preproduction inside definitive hosts (Sousa 1983, Galaktionov and Dobrovolskij 2003, Huspeni and Lafferty 2004, Hechinger 2007).

Larval trematodes are almost always specific to their molluscan first intermediate hosts, which are often dominant consumers within a habitat, and have less specificity to downstream hosts; some trematode species use a variety of vertebrate and invertebrate second intermediate hosts. Trematodes can account for huge proportions of total ecosystem biomass: larval trematode biomass equaled or exceeded that of dominant insect orders in a study of three California ponds, and comprised 18-33% of snail host biomass (Preston et al. 2013). Similarly, in three southern California and Baja California estuaries, larval trematode production in snail hosts alone exceeded the winter maximum of bird biomass, while total parasite biomass was greater than that of all top predators combined (Kuris et al. 2008).

Like many parasite taxa that infect multiple hosts, trematodes often alter morphology and behavior of intermediate hosts in ways that facilitate transmission to subsequent hosts (Esch and Fernandez 1994, Sorensen and Minchella 2001), with important ecological effects. In the California estuaries studied by Lafferty et al. (2006), trematodes add 977 food web links, at least one of which is quite dramatic: metacercariae of *Euhapsis chloriensis*, the most abundant trematode, manipulates killifish to increase predation by definitive bird hosts by 30 times (Lafferty and

Morris 1996) by altering the fish's behavior via serotonin and dopamine pathways (Shaw and Øverli 2012).

Trematode impacts on snail hosts

Trematodes often cause gigantism in snail hosts, inducing hosts to resume growth after sexual maturation and to grow at accelerated rates (Baudoin 1975, Sorenson and Minchella 2001, Miura et al. 2006b, Hechinger 2010). Host gigantism should be advantageous for the parasites, since cercarial size and production increase with host shell length (Poulin 2006). Alternatively, trematodes may stunt growth of snail hosts, as the developing parasites damage to digestive and other tissues (Sousa 1983, Curtis 1995, Mouritsen et al. 1999). Snails infected by trematodes may graze more or less algae, influencing benthic community structure (Bernot and Lamberti 2008,Wood et al. 2007).

Trematodes may modify the behavior of infected snails, inducing hosts to move to higher or lower tidal elevations relative to uninfected snails (Curtis 1987, Miura 2006b). These distributional changes are likely to affect how the host interacts with its food resources, leading to potential top-down effects on benthic communities. Because snails are often the dominant invertebrate consumer within a habitat, it is important to understand both the nature and extent to which trematodes alter their morphology and behavior, and how these changes affect resource use. This is particularly true in estuaries and other aquatic systems that experience eutrophication and associated algal blooms and, often, hypoxia, as snails may exert important

controls in these systems (Silliman and Zieman 2001). It is likely that trematode distributions, abundances, and interactions with hosts are expected to change with increasing global temperature and eutrophication (Marcogliese 2008), so these systems are good models for studying trematodes' effects on both hosts and ecosystem processes.

This study characterizes the effects of trematode parasites on the morphology and behavior of a non-native mud snail, *B. attramentaria*, in Elkhorn Slough, a central California estuary that is highly impacted by agricultural nitrogen and associated eutrophication (Hughes et al. 2011). *Batillaria* is a highly successful invader of intertidal habitats in estuaries from Boundary Bay, BC (Canada), to Elkhorn Slough (Watsonville, CA). It is typically the dominant intertidal mudflat invertebrate, and reaches densities exceeding 22,000 individuals/m^2 (unpublished data) in Elkhorn Slough. *Batillaria* displaces the native snail, *Cerithidea californica,* and the ~18 species of trematodes that use *Cerithidea*, where the snails have overlapped. In its native range, *Batillaria* is parasitized by at least 9 morphologically distinct trematode species (Hechinger 2007), and by only one species complex, *Cercaria batillariae,* in its introduced range. This species complex is composed of 8 cryptic species, two of which are common in the introduced range (Miura et al. 2006a). *C. batillariae* uses fish as second intermediate hosts (Shimura and Ito 1980, Hechinger 2007), but its avian definitive hosts are not known.

Miura et al. (2006b) showed that *Batillaria* infected with *C. batillariae* in its native range in Japan resume growth after maturation, and grow faster than

uninfected snails. Infected snails also moved to lower tidal elevations, presumably to facilitate transmission to subsequent fish hosts. We compare *C. batillariae*'s effects on *Batillaria* in Japan to its effects in the species' introduced range, where *C. batillariae* is the only trematode species using *Batillaria*. Elkhorn Slough is an opportune system for studying these interactions. *Batillaria* are both abundant and highly parasitized in this nutrient-impacted system (Lin 2006, data presented here), so any trematode effects on snails are likely to impact benthic dynamics in ways that could be ecologically important.

1.3 Methods

Mark-recapture growth experiment

A total of 522 *Batillaria* were collected opportunistically at Azevedo Channel (Figure 1) on June 2, 2014 along ~10 m of intertidal mudflat and taken to the Long Marine Laboratory. Their shells were rinsed with fresh water and scrubbed with stainless steel brushes before being marked with enamel paint markers at the aperture and apex of each shell. Shell lengths were measured to the neared 1mm using calipers. Snails were returned to the collection site on June 4, and recovered on August 1, 2014. Snails were rinsed and frozen, and shell length, width at aperture, and shell extension were measured to the nearest 0.1 mm using calipers. Snails that had measurable growth were mounted vertically underneath a dissection microscope, with the growing body whorl facing up. Needles were aligned with the radii of the original and new apertures of the growing body whorl, and snails were photographed.

Angular growth was measured as the angle between new and old apertures using ImageJ software (after Miura et al. 2006b, see Figure 2). Angular growth rather than shell extension corrects for differences in snail width. Snails were then dissected, and determined to be either infected or uninfected with trematode rediae. Infected snails were assigned to three categories based on abundance of parasite rediae: Low = less than one-third of gonad/digestive tissue occupied by rediae; Medium = one-third to two-thirds of gonad/digestive tissue occupied by rediae; High = over two-thirds of the gonad/digestive tissue occupied by rediae.

Transect surveys

Eight transects, running from high to low tidal elevation, were conducted along the North Marsh channel at Kirby Park (Watsonville, CA, Figure 1) on May 8 and May 11, 2014, using a random number generator to assign the number of paces between transects. Between 6 and 12 stations were established along each transect at evenly spaced tidal elevations. The first station of each transect was at the marsh edge, determined by the lower limit of pickleweed (*Salicornia virginica*), and each subsequent station was 0.1m lower than the previous station, until no more snails were present. Because snails were patchily distributed, tidal elevations varied slightly across transects. Tidal elevations were determined from LiDAR-derived data (provided by Charlie Endris, GIS Specialist, Elkhorn Slough National Estuarine Research Reserve). At most stations, a metal cylinder (8.5 cm dia) was placed as close as possible to the desired tidal elevation, then all snails within the cylinder were

collected. At lower tidal elevations (below ~0.25 m above MLLW), snails were scarce, so all snails were collected within a 1m radius of stations.

Snails were taken to the Long Marine Laboratory, where they were rinsed with fresh water and frozen until they could be dissected. Before dissection, shell length was measured to the nearest mm with calipers. Snail infection status and abundance of rediae in infected snails were recorded.

Statistical Analyses

Mark-recapture

A Wilcoxon test, including all live snails that were recovered, was performed to assess whether shells of infected snails were longer than those of uninfected snails. Angular growth of snail shells was log-transformed to achieve normality before a one-way ANCOVA was run to determine whether growth rates of infected and uninfected snails were different. The ANCOVA used shell width as the covariate, and only included snails with measurable growth.

Transect surveys

Separate multiple linear regressions were performed to determine the effect of tidal elevation on individual shell length, proportion of snails infected within samples, and proportion of infected snails with high parasite abundances within samples. All multiple regressions included transect as an effect source, and the shell length

regression included infection status as an additional effect source. A Wilcoxon test was also performed to assess whether shells of infected snails were longer than those of uninfected snails. Samples with fewer than five snails were excluded from the multiple regression of proportion of snails infected, and samples with fewer than four infected snails were excluded from the regression of parasite abundance. All statistical analyses were performed in JMP Pro 12.0.1.

1.4 Results

Mark-recapture growth experiment

We recovered a total of 296 live snails, 16 intact dead snails, and 10 fragments of snails that had been predated upon. Infected snails were significantly longer (29.59 ± 0.34 mm) than uninfected snails (22.89 ± 0.15 mm; $p < 0.0001$, Figure 3). Of the live snails recovered, 181 had measurable growth, 47 did not grow, and 68 could not be measured because the aperture marking from the beginning of the study was not discernable. Among snails that had measurable growth, 46% were infected – twice the rate of snails that did not grow (23.4%). Among snails with measurable growth, infected snails grew significantly more during the study period than uninfected snails (24.89° ± 1.15° and 19.71° ± 0.92°, respectively, $p < 0.0001$; Tables 1, 2, Figure 4).

Transect surveys

We collected a total of 340 snails along eight transects, 248 (72.9%) of which were infected. Shell length was inversely correlated with tidal elevation ($p < 0.0001$, Figure

5); shell length also varied among transects and was significant in the multiple regression. As in the mark-recapture study, infected snails were significantly longer than uninfected snails (25.33 ± 0.27 and 20.12 ± 0.45 mm, $p < 0.0001$, Table 3), and infection status significantly affected shell length in the multiple regression ($p < 0.0001$). Both the proportion of snails infected within samples and the proportion of infected snails with high parasite abundances were inversely correlated with tidal elevation ($p = 0.0062$ and 0.0057, respectively, Table 4, Figures 6,7).

Comparing sites, infection rates were much higher at Kirby Park (72.9%) than at Azevedo Channel (41.7%), while both infected and uninfected snails were significantly longer at Azevedo Channel than at Kirby Park ($p < 0.0001$, Wilcoxon test). Despite differences in prevalence of infection and shell lengths at the two sites, the relative pattern of infected snails being larger than uninfected snails was consistent between sites.

1.5 Discussion

Parasite-induced changes to snail growth

Batillaria infected by *C. batillariae* were larger than uninfected snails in both the mark-recapture study at Azevedo Channel, and in the transect surveys at Kirby Park. Infected snails were 29.3% and 25.9% longer than uninfected snails at Azevedo Channel and Kirby Park, respectively, similar to the 20-30% differences in shell length reported by Miura et al. (2006b) in Japan. Infected snails also grew more than uninfected snails during the mark-recapture experiment, confirming that the

correlation between snail size and infection status is not simply a function of parasites selecting larger snails to infect.

Accelerated growth of infected snails is in marked contrast to most studies of trematode-infected marine snails, in which the parasites either stunted or did not affect snail growth (Sousa 1983, Lafferty 1993b, Curtis 1995, Mouritsen et al. 1999, Bordalo et al. 2014). Instead, our results are similar to studies showing enhanced growth of freshwater snails infected by parasitic castrators, some dating back many decades (reviewed in Baudoin 1975, Sorensen and Minchella 2001). The absence of enhanced growth in parasitized marine snails has generally been attributed to marine snails' allocating relatively little energy to reproduction, since this effort is spread over a longer life span, and the energy conserved by truncated reproductive costs is sufficient only for repairing damaged tissues and supplying the parasites' nutritional requirements (Sousa 1983). Of the ten studies of seven species of trematode-infected marine snails reviewed by Sorensen and Minchella (2001), only one found increased growth of infected snails. That study investigated the effects of trematode infection on three snail species, and found increased growth in only one, *Onoba aculeus* (Gorbushin and Levakin 1999). The authors attributed this disparity to *O. aculeus*' short lifespan (2-3 yr) relative to the other species studied (9-11 yr), citing the mechanism described above.

Miura et al. (2006b) were the first to report trematode-induced gigantism in a long-lived marine snail (*Batillaria attramentaria* in Japan), using a mark-recapture experiment and transect surveys. Hechinger (2010), working with a guild of

trematodes species infecting *Cerithidea californica*, showed that both parasites and uninfected snails allocated energy to growth or reproduction based on the likelihood of extrinsic mortality (i.e. being killed by means other than senescence), and infected snails grew more than uninfected snails. The probability of extrinsic mortality varies among the ~18 species of trematodes that use *Cerithidea*, based on their positions within a stable hierarchy (dominant parasites outcompete or kill subordinate species), or, for subordinate species, having strategies to avoid mortality (e.g. tolerating co-infection with other low- and mid-ranking species; occurring only in geographic locations where dominant species are absent). Trematodes with low extrinsic mortality allocated more energy to the growth of the snail bodies they occupied than to their own reproduction; conversely, parasite species with high extrinsic mortality devoted more energy to reproduction than to the growth of snail bodies. Uninfected snails, facing imminent reproductive death (castration), also allocated more energy to reproduction than to growth; this is consistent with findings that *Cerithidea* matures at smaller sizes in populations with high parasitism rates (Lafferty 1993a). Overall, snails infected with trematode species with low extrinsic mortality grew the most, while uninfected snails and snails infected by trematodes with high extrinsic mortality grew the least, allocating energy to reproduction instead. Since there is such a range of parasitic effects on *Cerithidea*'s growth based on differential mortality, is not surprising that earlier investigators did not observe increased growth of infected *Cerithidea.*

Because *Batillaria* is potentially long-lived (8-10 yr, Yamada 1982), increasing snail size should be advantageous for the parasite in two ways: larger size reduces the likelihood that infected snails will be predated upon (Sousa 1993), and it leads to increased production, size, and/or viability of cercariae (Poulin 2006). It is unknown whether parasite-induced gigantism is as common in marine snails as it is in freshwater snails, since so few studies have appropriate data on growth and infections. We suspect that there may be more cases of trematode-induced gigantism in marine snails than have been reported, particularly in food-replete environments.

It is possible that marine snail hosts tend to be more food-limited than freshwater snails, decreasing their potential for increased growth. Alternatively, marine snail hosts may suffer more tissue damage from trematode infections than freshwater hosts, making increased growth less likely. For example, Wood et al. (2007) surmised that decreased grazing by trematode-infected *Littorina littorea* was at least partially caused by impairment of digestive tissue caused by the infection. Tissue damage can be exacerbated by other stressors, such as hypoxia (Sousa and Gleason 1989), which could limit growth, or kill hosts before they can reach larger sizes. It has also been suggested that hosts that have grown to large sizes, harboring high abundances of trematodes, are more susceptible to other stressors. For example, higher temperatures might kill large hosts due either to further temperature-driven increases in trematode production, or to increased virulence of existing loads (Poulin 2006, Marcogliese 2008). Given that both warming and hypoxia are expected to

increase in coastal waters, more studies are needed to explicitly address the impacts of these changes on trematodes and their snail hosts.

Parasite infection along tidal gradient

In the transect surveys, the proportions of snails that were infected were greater at lower tidal elevations, supporting our hypothesis that parasites induce movement of infected snails to lower tidal elevations. The proportion of infected snails with high abundances of active rediae was also greater at lower tidal heights, indicating that parasite loading is an important mediator of the magnitude of parasitic effects, as has been seen in other studies (Poulin 1994, Lafferty and Morris 1996).

We attribute these results to *C. batillariae* having adaptations that modify *Batillaria*'s behavior in ways that increase transmission to fish hosts. Although movement to lower tidal elevations could be linked to increased snail growth, since snails feed only when submerged, this is unlikely, because *Batillaria*'s primary food source, benthic diatoms, are less abundant lower in the intertidal (Fabian, unpublished data). Movement of infected snails to lower tidal elevations could also be an adaptation of the host, alleviating competition with uninfected snails. It is difficult to show that parasites directly manipulate host behavior, as changes may be accidental byproducts of infection, effects of host defenses against parasites, or adaptations inherited from ancestors of the parasites (Thomas et al. 2005). Because castrated hosts have experienced reproductive death, any effects on host morphology or

behavior that increase survival and/or transmission to subsequent hosts will increase the fitness of the parasite and are likely to be selected for.

Most published examples of trematodes affecting intermediate host distributions within a habitat have documented facilitated predation on intermediate hosts by subsequent hosts (e.g. Bakker et al. 1997, Thomas and Poulin 1998), while studies documenting increased non-feeding cercarial transmission are less common. Curtis (1987) showed that trematode-infected *Ilyanassa obsoleta* became stranded on beaches or sand bars during high tides, a behavior likely to enhance transmission of free-swimming cercariae to the semi-terrestrial crabs that serve as its second intermediate host. Similarly, Miura et al. (2006b) showed that *Batillaria* infected with *C. batillariae* moved lower in the intertidal zone, which increases time spent submerged, a prerequisite for cercarial transmission to fish hosts.

Contrasts with trematode effects in Batillaria's native range

While our conclusions are similar to those of Miura et al. (2006b), some differences between the studies suggest some insights into how ecological and environmental conditions mediate parasitic manipulation of hosts. In our transect surveys, infected snails tended to be distributed at lower tidal elevations, but both infected and uninfected snails were found across the intertidal elevations surveyed. This is in stark contrast to Miura et al.'s results, where there was virtually no overlap between snails infected with *C. batillariae* and uninfected snails. One possible explanation is that a genetic bottleneck reduced the diversity of haplotypes of cryptic

species of *C. batillariae* and/or of *Batillaria* itself, so that the host-parasite interaction has a different effect on *Batillaria*'s behavior in Elkhorn Slough than in its native Japan. Although the dominant snail haplotype is the same in both places, the dominant cryptic species of *C. batillariae* in Elkhorn Slough (HL6), which comprises ~76% of *C. batillariae* infections, accounts for only ~16% of *C. batillariae* infections in Matsushima Bay (Miyagi Prefecture) (Miura et al. 2006a). HL6 was introduced with *Batillaria*, and its genetic differentiation from source populations in Japan indicates that it is not continuously dispersed by migratory birds, unlike the other cryptic species (HL1) that is common in the introduced range (Miura et al. 2006a). Thus, contemporary HL6 infections in Elkhorn Slough probably originated in *Batillaria*'s introduced range, since the time since *Batillaria*'s introduction far exceeds the maximum lifetime of the snail. While neither our study nor Miura et al. (2006b) differentiated among cryptic species of *C. batillariae*, the different in relative abundances of cryptic species, and the evidence for genetic differentiation between introduced and source HL6 Japan populations, could contribute to the different behavior and spatial distributions of infected snails in Matsushima Bay and Elkhorn Slough.

Another explanation for these differences is that *C. batillariae* in Elkhorn Slough are released from competition with other trematode species (and/or other cryptic species of *C. batillariae*) – which, in Japan, use various molluscs, polychaetes, fishes, and birds after *Batillaria* (Hechinger 2007). Effects of different trematode species on *Batillaria*'s position in the intertidal in Japan are averaged when

snails are infected by two species (Miura and Chiba 2007), suggesting that trematodes compete for expression of their effects on snail hosts. If altering host behavior is metabolically expensive for the parasite, these effects are likely to be diminished in the introduced range, where *C. batillariae* is the only trematode using *Batillaria.* Trematodes affect snail host physiology and/or behavior through the combined effects of excretory/secretory (E/S) products and alteration of gene expression in host immune and neuroendocrine systems; these processes are complex, with both direct and indirect components (de Jong-Brink et al. 2001). Since the exact mechanisms by which *C. batillariae* impacts *Batillaria* are unknown, we cannot speculate as to whether *C. batillariae*'s effects on *Batillaria* are costly.

A third possibility is that *Batillaria* is more susceptible to subtidal predators in Elkhorn Slough than in Japan, such that snail movement to lower tidal heights increases extrinsic mortality of both host and parasite. Crabs in Elkhorn Slough do eat *Batillaria* at least on occasion, even very large *Batillaria* (> 30 mm); since most observations of predation were at low tidal elevation (Fabian, unpublished data), it is conceivable that predation pressure is affecting *Batillaria*'s distribution. Snail size significantly increased with decreasing tidal elevation in our study, even when controlling for infection status, unlike in Miura et al.'s (2006b) study, where the correlation between snail size and tidal elevation disappeared when controlling for infection status. This suggests that factors other than trematode infection govern *Batillaria*'s size distribution in Elkhorn Slough; predation pressure is one potential explanation for this, based on our observations of crab predation on *Batillaria*.

Whether any of these mechanisms explain the differences in effects of *C. batillariae* on *Batillaria* in Japan and in Elkhorn Slough would require more extensive study, but it is clear that effects of trematodes on their snail hosts are context-dependent and are likely to vary in space and time. Many calls have been made for inclusion of parasites in food webs (Marcogliese and Cone 1997, Lafferty et al. 2008) because parasites are involved in so many interspecies interactions (Lafferty et al. 2006) and form large components of total ecosystem biomass (Kuris et al. 2008, Preston et al. 2013). It is important that undertakings to include parasites in food web models consider that effects of parasites on hosts, even of the same species, are likely to vary at different locations.

Implications for ecological interactions in Elkhorn Slough

Snails are often the most abundant macrofaunal consumers in aquatic and coastal marine environments, and their grazing can shape the structure (Lubchenco 1980) and production (Silliman and Zieman 2001) of benthic communities. They are commonly infected by trematodes that can increase (Bernot and Lamberti 2008) or decrease (Wood et al. 2007, Clausen et al. 2008) grazing by snail hosts, resulting in shifts in benthic algal biomass and community structure. *Batillaria* has been recorded at extremely high densities (>22,000 indiv/m^2) in Elkhorn Slough (Fabian, unpublished data), abundances likely to have measurable ecosystem effects.

Since we have shown that *C. batillariae* induces gigantism in *Batillaria* in Elkhorn Slough, we expect parasites might intensify grazing by snails. Larger

Batillaria tend to eat larger diatoms (Sousa 1983), and to consume greater quanitities of this food source (Byers 2000). Infected snails would probably need to eat more to fuel increased growth, unless the energy savings from castration is much greater than the energy diverted to parasite production. Many species, including shore crabs (Woolfolk and Labadie 2012) and many important macroinfauna (Wasson 2010) eat benthic diatoms in Elkhorn Slough, so increased grazing by snails could cause shifts in competitive interactions among species.

C. batillariae also induce snails to move to lower tidal elevations, where snails could be interacting differently with the benthic community than at higher tidal elevations. Miura et al. (2006b) found that infected snails, grazing at lower tidal elevations, had significantly different stable carbon isotope ratios, suggesting that snails grazing at high and low tidal elevations consume different types of food.

Whether snail migration to lower tidal elevations actually increases transmission to fish hosts, *C. batillariae* is very successful at infecting fishes in Elkhorn Slough: all adult fishes collected in one beach seine were infected with metacercarial cysts throughout their body cavities (staghorn sculpin, *Leptocottus armatus*; longjaw mudsucker, *Gillichthys mirabilis*; arrow goby, *Clevelandia ios*) (Torchin et al. 2005). Effects of *C. batillariae* on these fishes, which are common in the slough, are unknown. Whether *C. batillariae* manipulates fish hosts to enhance predation (similarly to *E. chloriensis*' manipulation of killifish, described in the introduction) is likely to depend on the feeding behavior of the definitive avian host(s). Predation enhancement can also lead to reduced fitness for the parasites

because manipulated hosts may be eaten by predators that are not suitable hosts, resulting in the deaths of host and parasite alike (Mouritsen and Poulin 2003).

Effects of parasites on *Batillaria* and associated trait-mediated indirect effects on ecological interactions vary in space and time. Infection rates can vary greatly over small distances: e.g., the prevalence of infection was nearly 60% higher at Kirby Park than at Azevedo Channel, less than a kilometer away. Both snail and parasite activity are seasonal; *Batillaria* is most active in summer months (Byers 1999), and trematode production occurs only from spring to late summer when water temperatures are high enough (~18°C minimum for trematodes infecting *Cerithidea*, Fingerut 2003). Thus we would expect parasite-mediated ecological effects to be greatest in the summer, but more studies are needed to get a better idea of how parasitic manipulation of snail hosts vary seasonally within a system. It is critical, however, to take into account the role of trematode parasites in shaping size and habitat use of this extremely abundant mud snail.

Conclusions

C. batillariae induces its snail host, *Batillaria attramentaria*, to grow to larger sizes and to move to lower tidal elevations. These effects are likely to be beneficial to the parasite by increasing parasite production, size/viability, and transmission to subsequent fish hosts, even if these effects are not direct adaptations for the parasite. Our findings are similar to those of Miura et al. (2006b) in *Batillaria*'s native range in Japan, but our findings differ in ways that indicate ecological context is important in

mediating effects of trematodes on snails. More studies are needed to quantify the ecological implications of trematode-induced changes to snail hosts, and to characterize spatiotemporal variation of these effects, particularly for non-native host-parasite interactions that have been subject to bottleneck effects. It is critical to account for effects of parasites on abundant snail hosts in estuarine systems, and potential food web effects, in order to manage these important habitats, particularly in the face of increasing global change.

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1.7 Figures and Tables

Figure 1: Transect survey and mark-recapture study sites

Figure 1: Azevedo Channel, site of mark-recapture study, and Kirby Park, site of transect surveys. Sites are ~350 m apart.

Figure 2: Method for measuring snail angular growth

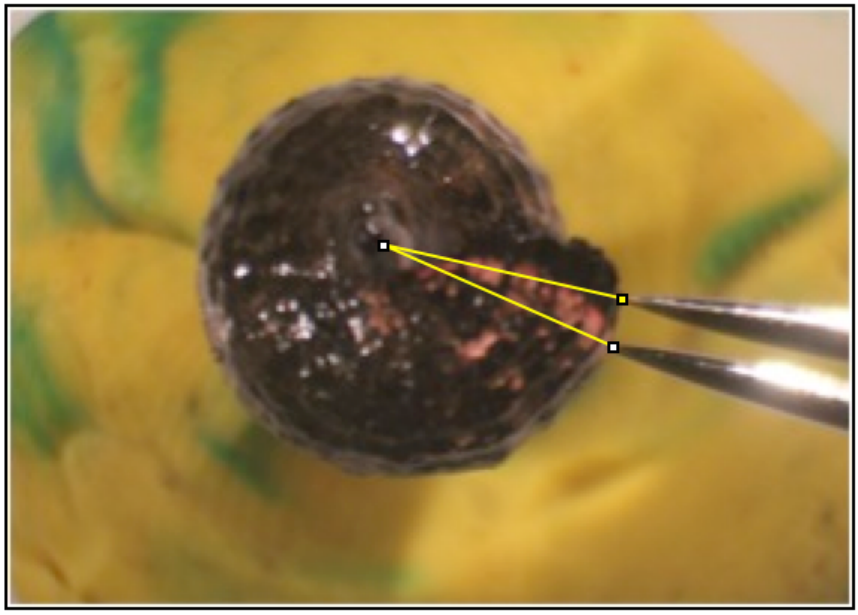

Figure 2: Measuring angular growth. Snails were mounted vertically and photographed underneath a dissecting microscope with needles pointed at the aperture marking from the beginning of the study and the aperture at the end of the study. Growth was measured as the angle between the needles and the center of the growing whorl using ImageJ.

Figure 3: Shell length of infected and uninfected snails, Mark-recapture experiment

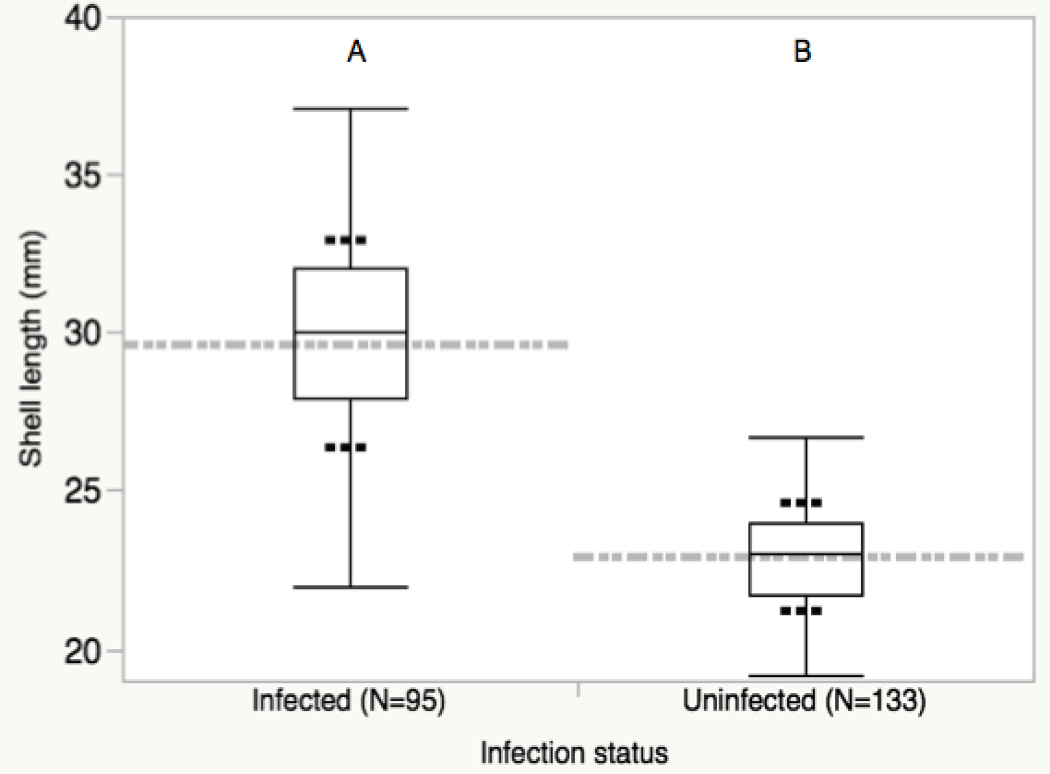

Figure 3: Quantile box plots of shell lengths of all live infected and uninfected snails recovered on August 1, 2014. Dashed lines are mean shell lengths and standard deviations. Whiskers extend to 1.5*(interquartile range) from the first and third quartiles. Letters indicate a statistically significant difference in shell length ($p <$ 0.0001, Wilcoxon test).

Figure 4: Snail angular growth, Mark-recapture study

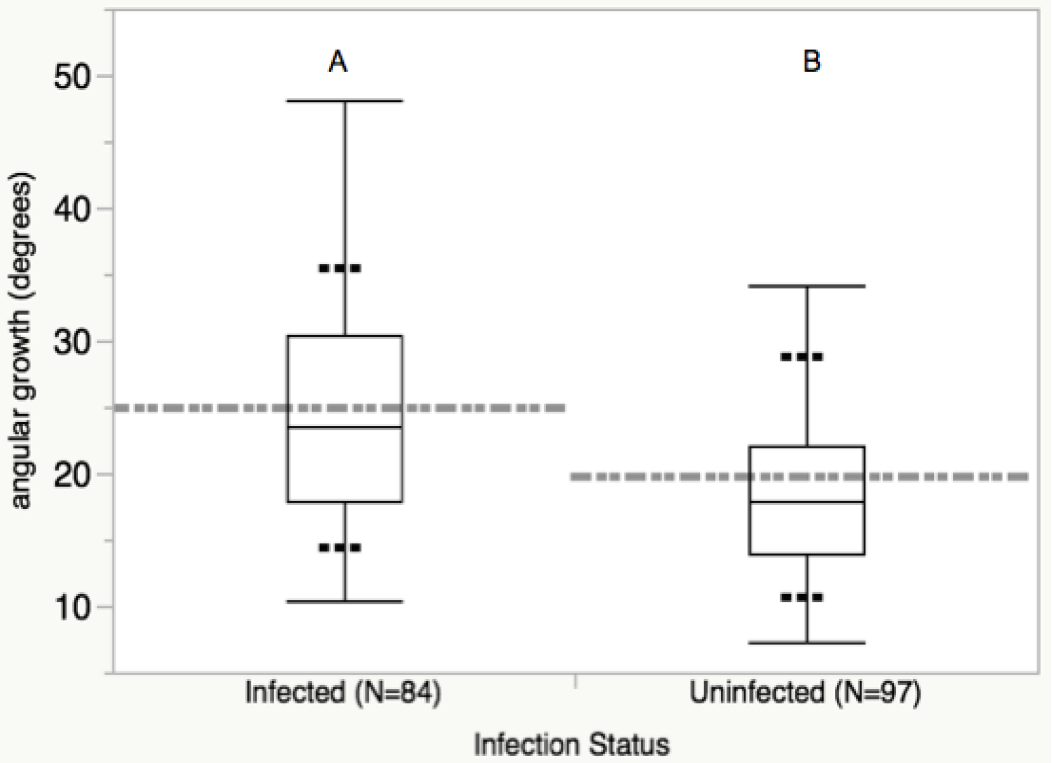

Figure 4: Quantile box plots of angular growth of infected and uninfected snails that had measurable growth from June 4 to August 1, 2014. Dashed lines are mean angular growth and standard deviations. Whiskers extend to 1.5*(interquartile range) from the first and third quartiles. Letters indicate a statistically significant difference (ANCOVA $p < 0.0001$, Table 2). For analysis, data were log-transformed to achieve normality; raw data are presented here.

Figure 5: Snail shell length and tidal elevation, Transect surveys

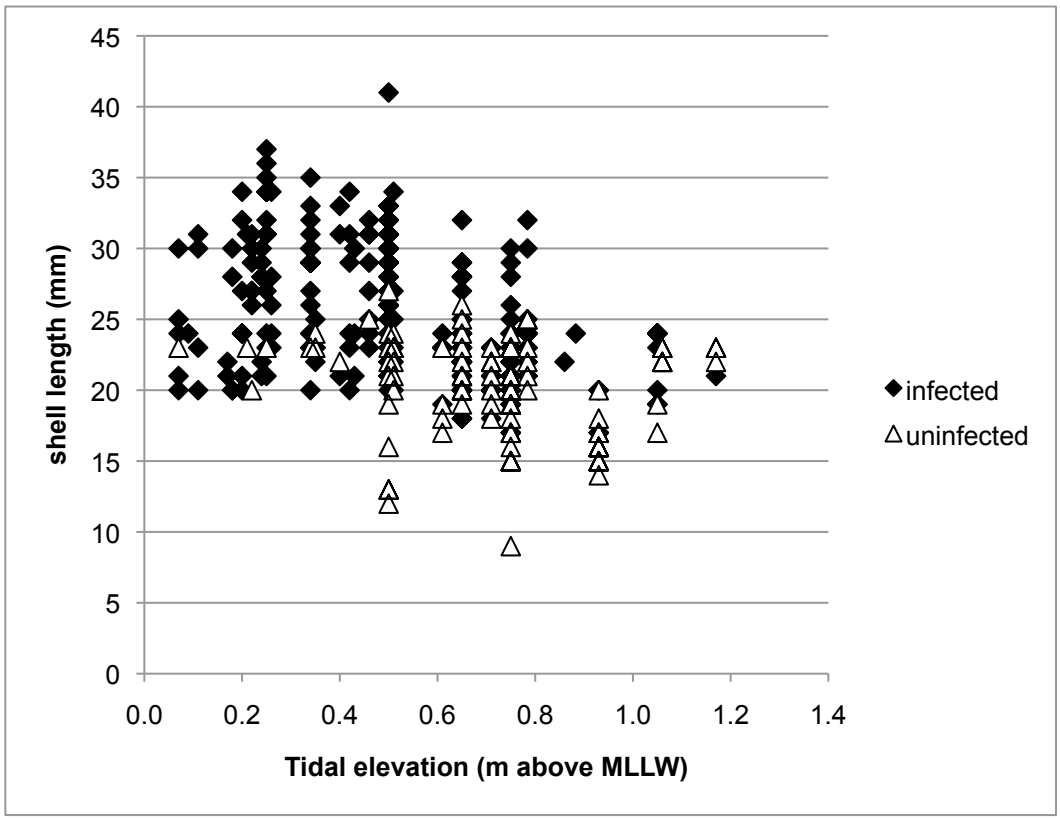

Figure 5: Shell lengths of infected and uninfected snails plotted against tidal elevation.

Figure 6: Proportion of infected snails by tidal elevation

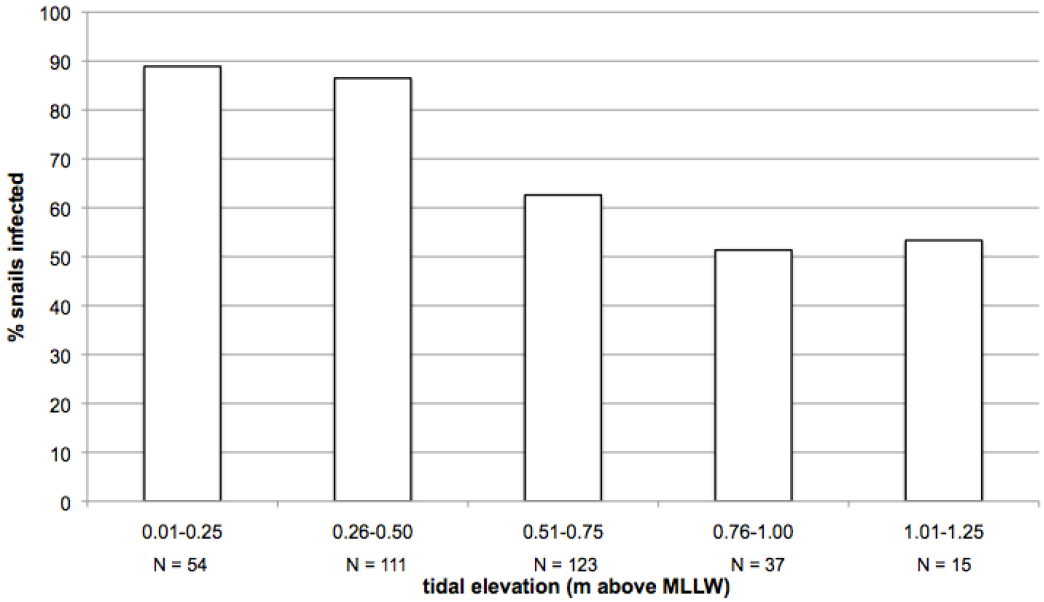

Figure 6: Percentages of infected snails, grouped into 0.25m tidal elevation bins. Samples from all transects were combined to yield one value for each bin.

Figure 7: Parasite abundance in infected snails

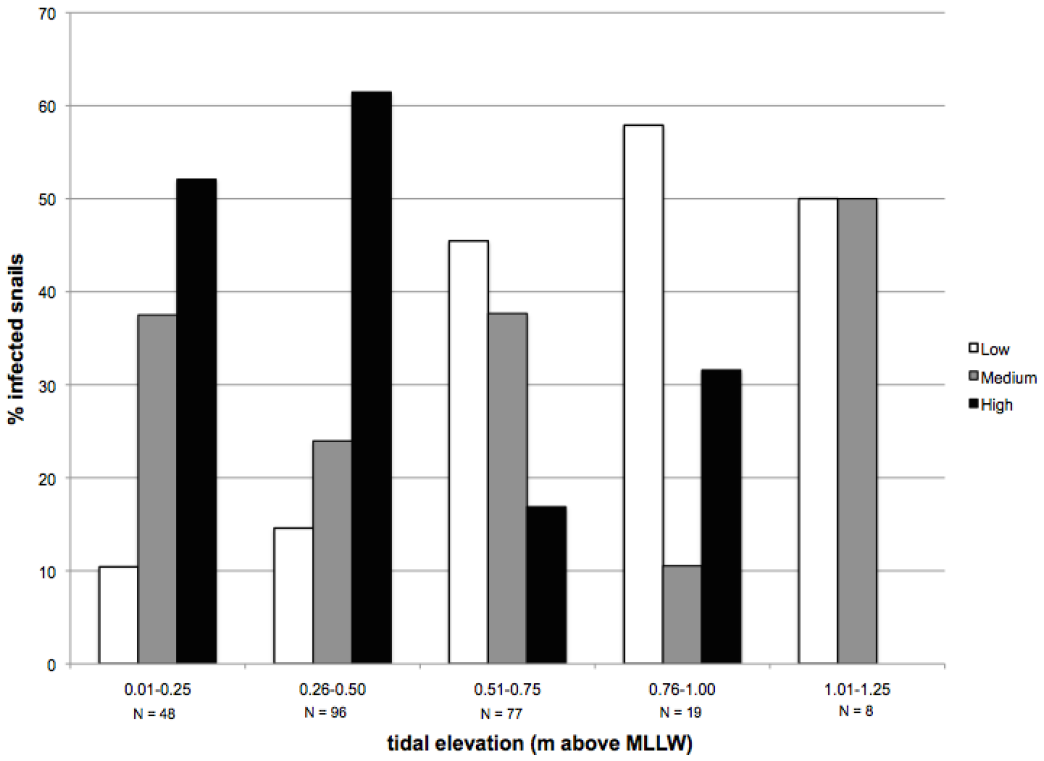

Figure 7: Percentages of infected snails with low, medium, and high parasite abundances, grouped into 0.25m tidal elevation bins. Samples from all transects were combined to yield one value for each combination of bin and parasite abundance designation.

Table 1: Snail sizes and growth measurements in the mark-recapture study

	Snails with measurable growth			Snails with no growth	
Infection status	Mean length ± SE (mm)	Mean width ± SE (mm)	Mean angular growth ± SE (°)	Mean length ± SE (mm)	Mean width ± SE (mm)
Infected	29.61 ± 0.36	9.97 ± 0.10	24.89 ± 1.15	29.46 ± 0.92	10.05 ± 0.30
Uninfected	22.94 ± 0.17	8.75 ± 0.07	19.71 ± 0.92	22.74 ± 0.30	8.52 ± 0.11

Table 1: Mean shell sizes and growth measurements for snails in the mark-recapture study.

Table 2: Summary of ANCOVA Results of snail angular growth in the mark-recapture study

Effect source	DF	SS	F-ratio	p
Infection Status	1	0.7372	25.61	<0.0001
Shell width	1	0.2558	8.88	0.0033

Table 2: Results of analysis of covariance of snail angular growth, with snail infection status and shell width as effect sources. Angular growth was log-transformed to achieve normality, and only snails that grew were included in the analysis.

Table 3: Mean shell length

Study/Location	Infection status	N	Mean length ± SE (mm)
Transects/Kirby Park	Infected	248	25.33 ± 0.29
Transects/Kirby Park	Uninfected	92	20.12 ± 0.37
Mark-recapture/Azevedo Channel	Infected	95	29.59 ± 0.34
Mark-recapture/Azevedo Channel	Uninfected	133	22.89 ± 0.15

Table 3: Mean shell lengths of snails from both the transect surveys and mark-recapture studies.

Table 4: Multiple linear regression analysis of relationships between tidal level and infection metrics

Response	N	Coefficient	F ratio	p	R^2
Shell length	340	-7.13	60.82	<0.0001	0.45
% Snails infected	29	-0.52	9.35	0.0062	0.58
% High parasites	24	-0.95	10.36	0.0057	0.52

Table 4: Results of three multiple linear regressions analyzing effects of tidal level on shell length, proportion of snails infected per sample, and proportion of infected snails per sample with high parasite loads. All models included transect as an effect source; the analysis of shell length included snail infection status as an additional effect source. For shell length, N is the number of individuals; for proportion of infected snails and proportion of infected snails with high parasite abundances, N is the number of samples included. Samples with fewer than five snails were excluded from the proportion of snails infected, and samples with fewer than four infected snails were excluded from the high parasite abundance regression. R^2 include all effect sources.

Chapter 2: Effects of parasite-induced changes to snail hosts on their microphytobenthic resources

2.1 Abstract

The trematode (fluke) parasite *Cercaria batillariae* causes its estuarine mud snail host, *Batillaria attramentaria*, to grow larger and more rapidly, and to move to lower elevations on intertidal mud flats to increase transmission to fish hosts used by the next life stage of the parasite. Snails suffer reproductive death when infected because the parasites consume and inhabit snail gonad tissue, and snails do not recover from infections. The cessation of snail reproduction should provide energy to support the snails' increased growth, but infected snails have the added energetic costs of parasitic production and repair of damaged tissues. These costs likely require infected snails to increase consumption of their primary food, benthic diatoms, to support this rapid growth. Together with cyanobacteria, diatoms comprise the microphytobenthos (MPB), the assemblage of algal cells growing in the upper few millimeters of illuminated aquatic sediments. The MPB provides integral ecosystem functions in shallow aquatic environments: they increase sediment stability by secreting extracellular polymeric substances (EPS) that glue sediment particles together, and by supporting estuarine food webs, where MPB can account for half of total primary production. *Batillaria* is the dominant macrofaunal grazer on mud flats in Elkhorn Slough, a central California estuary where the snail was accidentally introduced on oysters stocks from Japan. The snail occurs in densities of several thousand individuals per m^2 at some sites, so increases in diatom consumption by infected snails would be likely to affect the MPB (diatom size distributions, chlorophyll a)

and/or sediment chemistry (TKN, organic matter). In a 10 week caging experiment, we tested the hypothesis that large, infected snails have different effects on the MPB and sediment chemistry than small, uninfected snails, or control treatments with no snails. We deployed cages at two tidal elevations in order to characterize the effects of infected snails both at the mid-high elevations that are typically inhabited by snails, and at lower elevations that uninfected snails do not tend to occupy. At the high elevation, large (infected) snails doubled benthic chlorophyll *a*, a proxy for MPB biomass, relative to small (uninfected) snails and controls, while at low elevation, large (infected) snails reduced chlorophyll *a* to just ~25% of the levels in control treatments. Large (infected) snails also shifted the size distributions of diatoms inside cages, reducing the abundance of the largest cells. Because large diatoms are important contributors to estuarine food webs and sediment stability, the differential effects of infected and uninfected snails due to the effects of the parasites on snails are important to consider in management of estuarine systems.

2.2 Introduction

Studies of primary production in aquatic systems tend to focus on the roles of phytoplankton, macroalgae, or vascular plants in ecosystem function. However, the microphytobenthos (MPB), which appears as a green to brown film on seemingly unvegetated surfaces, can be an extremely important component of production in aquatic systems, providing up to 50% of estuarine primary production (Underwood and Kromkamp 1999). The MPB is composed primarily of diatoms and cyanobacteria that grow in the upper few mm of sediment in shallow aquatic habitats. Diatoms tend to dominate the MPB, especially on sand and mud flats, but their relative dominance shifts temporally at a given site (Lucas et al. 2001). Cyanobacteria and flagellates can be common in protected environments (MacIntyre et al. 1996).

The MPB moderates the exchange of nutrients between sediments and the water column, and it imparts stability to sediments via extracellular polymeric substances (EPS) produced by motile diatoms, and, to a lesser extent, by cyanobacteria. Diatoms alone have been reported to increase sediment stability by 120%, and stability increased by 150% in flume studies when cyanobacteria were also present (Lundvist et al. 2007). The MPB is attached to particles in exposed environments, where it interacts with clay and sand to create a gluing effect (Paterson 1989, Paterson et al. 1990, Tolhursf et al. 2002), and in protected environments it can form mats a few mm thick (MacIntyre et al. 1996).

The MPB can dominate primary production in intertidal environments (MacIntyre et al. 1996), where it is an important food source for many deposit and suspension feeders, including such benthic macrofauna and macroinfauna as snails, clams, and juvenile fishes (Miller et al. 1996, White and Haag 1977, Hughes et al. 2000). In addition to providing food resources, the MPB increases the abundance of infauna by reducing turbidity near the sediment/water interface (McClusky et al. 1993, Warwick et al. 1991).

Snails are often the most abundant macrofaunal consumers in aquatic and coastal marine environments, and their grazing can shape the structure (Lubchenco 1980) and production (Silliman and Zieman 2001) of benthic communities. On intertidal mud flats, snails graze on benthic diatoms, microorganisms, and detritus. As an example, burrowing and fertilization (via feces) by low densities of the mud snail *Ilyanassa obsoleta* can increase microalgal abundance, measured as chlorophyll *a*, by increasing nutrient availability, but at high snail densities, their grazing pressure exerted outweighs the positive effects (Connor and Edgar 1982). Burrowing by snails at high densities can also reduce MPB biomass by stirring sediments and inducing light limitation, particularly in depositional environments (Connor 1980).

Snails can impact the structure of the MPB directly by selective grazing on large, slow-growing diatoms, and thereby altering competitive interactions among its components (Connor 1980, Fenchel and Kofoed 1976). Snails may also have indirect effects on the MPB through interactions with infaunal species. Snail burrowing and grazing can negatively impact many infaunal taxa that also consume MPB (Hunt et al.

1987, Kelaher et al. 2003), and impacts to infaunal species can have additional trophic effects: e.g. high densities of *I. obsoleta* impact habitat use by shore birds through inhibition of the bird's preferred food, the amphipod *Corophium volutator* (Hamilton et al. 2003).

Snails in aquatic environments are commonly parasitized by trematode (fluke) parasites that infect and castrate their snail hosts by consuming the snails'gonads, later using one or more additional hosts (commonly invertebrates, fish, birds, and mammals) to complete their life cycles. Larval trematodes divide clonally within snails and other mollscan hosts, eventually developing into free-swimming forms (cercariae) that seek out and penetrate subsequent hosts. Snails do not recover from the infection, suffering reproductive death, but snail somatic tissues can survive for years, producing many parasite generations (Sousa 1983, Galaktionov and Dobrovolskij 2003, Huspeni and Lafferty 2004, Hechinger 2007).

Because trematodes commonly alter host morphology and/or behavior in ways that benefit the parasite, they are often referred to as manipulative parasites. Some snails that are infected by trematodes, including the marine snail *Batillaria attramentaria* (Miura et al. 2006b, Fabian, unpublished data) undergo gigantism, resuming and accelerating growth after maturation (reviewed in Baudoin 1975, Sorensen and Minchella 2001). Increasing host growth should be advantageous for the parasite, as larger snails produce larger (more viable), more abundant cercariae (Poulin 2006), and they are less likely to be predated upon (Sousa 1993).

Increased growth can be subsidized by diverting energy from truncated reproductive efforts, but the freed energy must also be allocated to parasite reproduction and repair of damaged host tissues. Thus it is likely that infected snails must eat more to fuel increased growth. Increased grazing by dominant snail consumers can intensify their effects on the MPB. For example, the freshwater snail *Physa acuta* grazes more when infected by trematodes, driving a shift in the MPB community from domination by cyanobacteria to *Cladophora*, a filamentous green alga (Bernot and Lamberti 2008).

Trematodes may also modify host behavior in ways that alter host distributions within a habitat. Infected snails move closer to the parasites' next hosts: *I. obsoleta* moves to higher tidal elevations, a behavior likely to enhance transmission of free-swimming cercariae to the semi-terrestrial crabs that serve as its second intermediate host (Curtis 1987), while *B. attramentaria* moves to lower elevations, increasing time spent submerged, a prerequisite for cercarial transmission to fish hosts (Miura et al. 2006b, Fabian, unpublished data). It is likely that infected snails growing at accelerated rates and occupying different tidal elevations interact differently with their microphytobenthic food resources. Miura et al. (2006b) reported infected *B. attramentaria* had lower $\delta^{13}C$ values than uninfected snails, suggesting that snail food intake was altered by parasites, but they could not discount other causes of the difference.

The present study explored the effects of parasite-induced gigantism and changes in the distribution of *Batillaria attramentaria* (hereafter, *Batillaria*) on the MPB and sediment properties in Elkhorn Slough, a central California estuary. *Batillaria* is a highly successful invader of intertidal habitats in estuaries from Boundary Bay, BC (Canada), to Elkhorn Slough (Watsonville, CA). It is typically the dominant intertidal mudflat invertebrate, and has reached densities exceeding 22,000 individuals/m^2 (Fabian, unpublished data) in Elkhorn Slough. Where their distributions have overlapped in the past, *Batillaria* displaces a native snail, *Cerithidea californica,* and ~18 species of trematodes that infect *Cerithidea*. In its introduced range, *Batillaria* is parasitized by only two members of one species complex, *Cercaria batillariae* (which is composed of 8 cryptic species, and is one of at least nine morphologically distinct species present in *Batillaria*'s native range in Japan, Hechinger 2007). *C. batillariae* uses fish as second intermediate hosts (Shimura and Ito 1980, Hechinger 2007), including staghorn sculpin (*Leptocottus armatus*), longjaw mudsucker (*Gillichthys mirabilis*), and arrow goby (*Clevelandia ios*) in Elkhorn Slough (Torchin et al. 2005). Its avian definitive hosts are not known.

Batillaria's primary food resource is benthic diatoms, and the snails often occur in such high densities that they can be expected to have an impact on the MPB in Elkhorn Slough. Infection by *C. batillariae* is widespread in Elkhorn Slough; about 46% of snails are infected slough-wide (Lin 2006), with infection rates as high as 73% at some sites (Fabian, unpublished data). Larger *Batillaria* tend to eat larger diatoms (Sousa 1983), and consume greater quanitities of them (Byers 2000). This

could impact other species, including shore crabs (Woolfolk and Labadie 2012) and other macroinfauna (Wasson 2010) that eat benthic diatoms, potentially leading to shifts in competitive interactions among species. Reduced diatom abundance could also lead to changes to sediment stability. This is of particular concern in Elkhorn Slough, because, since the dredging of Moss Landing Harbor in 1948, the slough has become an erosional system, with marsh area eroding to mud flats. The ebb-dominated tidal sediment transport results in a positive feedback, driving further increases in erosion (Breaker et al. 2008). Further increases to sediment erodibility due to alterations of the MPB could worsen the problem.

We present the results of a field caging experiment to assesses the impacts of both gigantism and altered distribution of *Batillaria* on the MPB and sediment properties in Elkhorn Slough. It is the first study to investigate both the impacts of increased growth and altered distribution of trematode-infected hosts. Because *Batillaria* is so abundant in Elkhorn Slough, we hypothesized that infected and uninfected snails have different effects on food resources, and, if they occur, are likely to be ecologically relevant.

2.3 Methods

Experimental design and set-up

The effects of parasitic-induced increases to *Batillaria*'s growth on their food resources and sediment characteristics were investigated using three snail treatments, with snail size serving as a proxy for infection status: small (≤ 22 mm, "uninfected");

large (≥ 30 mm, “infected”); and control (no snails). We deployed these treatments at two tidal elevations: high (0.9 m above MLLW) and low (0.3 m above MLLW) to compare the effects of parasitic infection at elevations densely inhabited by snails (high) and at elevations occupied mainly by infected snails (low). Eight experimental cages of each snail treatment were established at each tidal elevation along the north marsh channel at Kirby Park (Watsonville, CA, 36°50’26” N, 121°44’39” W). The small snail treatment at low elevation was discontinued because snails could not be retained in cages in sufficient abundances. Cages were sited along tidal elevation contours derived from LiDAR data (provided by Charlie Endris, GIS Specialist, Elkhorn Slough National Estuarine Research Reserve), and using a random number generator to determine the number of paces between cages. Snail treatments were randomized among cages by thoroughly shaking a bag of pre-marked garden tags and selecting tags randomly as cages were put in place.

A week before the experiment began, 400 large snails were collected from Kirby Park, and 400 small snails were collected from North Jetty Rd (36°49’01” N, 121°47’13” W), and taken to the Long Marine Laboratory at the University of California, Santa Cruz (UCSC) for processing in preparation for the experiment. Snails were rinsed with fresh water, scrubbed with stainless steel brushes, and marked at their apices with red (low cages) or green (high cages) enamel paint.

Cages were constructed from 1 x 1 mm plastic mesh wrapped around reinforcing wire mesh frames (~15 x 15 x 70 cm) that were attached with zip-ties to 1m-long vertical pieces of rebar. Cages were hand-driven ~10 cm into the sediment,

so that they extended ~60 cm above the mud and enclosed ~225 cm^2 of mud flat. Before cages were put in place, existing snails were removed from inside the areas to be caged, and the mud surface was rubbed to homogenize benthic diatom abundance within cages (after Byers 2000a). Cages were emplaced on July 1-2, 2014, and on July 4-5, 40 marked snails were added to each small snail and large snail treatment cage, resulting in snail densities of 1778 inidviduals/m^2, similar to abundances that are common in Elkhorn Slough (Fabian, unpublished data). The experiment ran for 10 weeks until September 9-10.

Cages were checked every ~2 weeks, depending on the simultaneous occurrence of daylight and tides low enough to access low cages. The cages did not have tops at the beginning of the experiment to avoid shading diatoms (after Byers 2000a, Armitage et al. 2009), but when cages were checked for the first time after 8 days, crabs had entered several of the low cages and a few of the high cages, and had consumed many small snails. The following day, all crabs were removed and hardware cloth tops (7.5 x 7.5 mm mesh) were added to cages the with zip-ties. The tops kept crabs out of the cages for the remainder of the experiment, except for a few small (carapace $\leq$ 2 cm) individuals, which were removed when cages were checked.

Snails were counted in each cage at the first two checks, and marked escapees were collected and returned to cages. Escapees were recorded as having moved up, down, or laterally in relation to the cages. After the second check (July 29-30), the small snail/low elevation treatment was abandoned because so few snails remained inside the cages. After the third check (August 4), snails were no longer counted to

avoid disturbing the mud surface. Instead, desired snail densities were maintained by adding snails to cages in numbers based on previous rates of loss. At the end of the experiment (September 9-10), cages were removed, snails were counted, and sediment samples were taken for subsequent analysis. All samples were placed on ice in a cooler immediately after collection and frozen at -20° C until they could be analyzed.

Benthic diatoms

Four 5 mm deep sediment samples were taken from each cage using a 1 cm diameter plastic syringe and pooled in a scintillation vial. Sediments were preserved in the field with 15 ml of 6% Lugol's solution in artificial seawater to stain chloroplasts in cells that were alive at collection. Samples were prepared for microscopy at UCSC's Marine Analytical Laboratory. Scintillation vials were mixed on a vortex mixer (Fisher Scientific) for one minute. A 350 µl subsample of the Lugol's solution-sediment slurry was mixed with 1 ml of deionized water and mixed for 30 seconds. 300 µl of the mixture was then pipetted onto glass slides with pre-marked with a 14.3 mm-diameter circles. Slides were allowed to dry overnight, and stored until they could be analyzed

Immediately before microscope analysis, a drop of Lugol's solution was placed on each slide to stain chlorophyll *a*, and a cover slip was added. Slides were viewed at 400x magnification under a Zeiss Axioskop, and all diatoms were counted and measured (maximum length) with an eyepiece reticle along four transects

radiating at 90° intervals from the pre-marked center of the circle to the edge. Transects were 7.15 mm long and 200 μm wide. Two slides were analyzed for each cage.

Chlorophyll a

Two 5 mm deep sediment samples were taken from each cage with a 1 cm diameter plastic syringe and combined in a 15 ml centrifuge tube. Before analysis, 10 ml of methanol were added to the tubes, and tubes were thoroughly shaken. After 6 hours, the samples were shaken again and centrifuged for 5 minutes. 1 ml of the methanol solution was then pipetted into a spectrophotometry cuvette (1 x 1 x 4 cm) with 2 ml of 90% ethanol. Optical densities were read at 665 nm and 750 nm using a Shimadzu UV-2101 Spectrophotometer. The samples were then acidified with 4 drops of 1M HCl and optical densities at 665 nm and 750 nm were read again after two minutes. Chlorophyll *a* concentration was calculated using Lorenzen's (1967) equation and converted to concentration per unit area (mg/m^2). Samples were protected from light throughout processing.

Sediment nitrogen and organic matter

We used a 2.6 cm diameter plastic syringe to collect two 5 cm deep samples for sediment nitrogen and two for organic matter analyses. Samples were pooled into Whirlpaks, and kept on ice in a cooler before being frozen at -20° C. Samples were dried at 55° C for five days, andinfaunal organisms and pieces of plant matter and

shells were removed. Sediments were then ground in a Wiley mill fitted with a #20 filter.

We analyzed nitrogen content using standard procedures for Total Kjeldahl Nitrogen (TKN), a measure that combines organic nitrogen and nitrogen as ammonia. ~400 mg of sediment were added to digester tubes with 3 acid resistant boiling chips and a Kjeltab. 10 ml of 4.5% H_2SO_4 was added to each digester tube, and tubes were heated for 3 hours. After cooling, 40 ml deionized water was added and tube contents were thoroughly mixed. 10 ml of the solution was poured off into scintillation vials, and ammonia content was measured with a Lachat QuikChem 8000 Flow Injection Analyzer.

Organic matter content was measured by drying samples overnight at 55°C and weighed. Samples were then combusted at 360° for three hours and re-weighed. Organic matter was estimated as percent loss on ignition (LOI).

Statistical analyses

Diatom abundance and length were analyzed with generalized linear models (GLM; Nelder and Wedderburn 1972) using base R 3.3 (R Core Team 2016). GLMs were fit with negative binomial distributions (log link) to account for over-dispersion. Summary means and associated confidence intervals were estimated separately by regressing diatom counts and lengths (binned in 50 μm length intervals) on snail size and tidal elevation combinations. Predicted changes in abundance correlated with length class were estimated by regressing abundance on natural log length bin.

Differences in benthic chlorophyll *a*, SOM, and TKN levels among snail sizes and tidal elevations were summarized with whisker plots. Correlations among environmental responses to snail treatments were analyzed with a one-way multivariate analysis of variance (MANOVA; SAS 9.4). Significance of the first canonical correlation (CC) was evaluated with Wilks' Lambda ($\alpha = 0.05$). The overall variance explained by the first canonical correlation of treatment effects was reported as the R^2 of the linear combination. Structural canonical coefficients of the first CC (square root of the partial R^2, or correlation to first CC) were examined to estimate relative effects of the treatments among environmental components. Standardized coefficients were interpreted as the relative effect size of each response. Effect magnitude on of each individual environmental variable was estimated from the raw canonical coefficients. *A priori* contrasts were used to establish significant differences among effects of treatments of the environment.

2.4 Results

Effects of snails on diatoms

In every treatment, diatom sizes were strongly skewed towards smaller size classes (Figure 2). There also appeared to be a small secondary peak of larger diatoms (<500μ) in each of the small and control treatments that was virtually absent from the two large snail treatments.

Summary statistics

All abundance and length distributions belonged to negative binomial distributions. Means and standard errors calculated with GLMs show that values for both metrics varied among snail-elevation treatments (Table 1). At high tidal elevation, benthic diatoms were most abundant in control cages and least abundant in cages with small (uninfected) snails. At low elevation, diatoms were about twice as abundant in control cages as in the large (infected) snail cages (Table 1).

At both tidal elevations, mean diatom length was lowest in large snail cages (Figure 3). At high elevation, mean length in the large snail treatments was about half the mean lengths of diatoms in the small snail and both control treatments. At low elevation, mean diatom lengths were smaller in large snail treatments than in controls, and median diatom lengths were similar in large snail and control treatments (Table 1).

Abundance regressed on length

The effects of separate slopes for natural log length bin by treatment were significant ($p < 0.001$) and all were negatively correlated (Table 2). Diatom sizes unilaterally were negatively correlated to length class and strongly skewed towards smaller size classes (Figure 2). There were two clear clusters, with large snail treatments statistically equivalent with much fewer diatoms present, especially for large length bins. The small snail treatment was not observably different from the control. The much steeper slopes for the two large snail treatments show that these

snails largely eliminated larger diatoms at both tidal elevations during the 10 week experiment.

Effects of snails on sediments

Chemical properties of the sediments in each treatment are summarized in Table 3 and illustrated in Figure 4. For chlorophyll *a*, the greatest difference was between the two large snail treatments: mean chlorophyll content for large snails at high elevation was almost an order of magnitude greater than at low elevation, with the controls and small snail treatments having similar, intermediate concentrations (Table 3, Figure 4). TKN and SOM were more variable (larger CVs in Table 3) with a different (but similar) ranking of treatments: mean concentrations in the two controls and the large snail low elevation treatment were about 50% greater than in the two high elevation snail treatments (Figures 5,6)

In the MANOVA, the simultaneous effect of snail and tidal elevation on chlorophyll *a*, TKN, and SOM was highly significant (Table 4; $p < 0.001$), with the first canonical correlations (CC) explaining most of the variation ($R^2 = 0.688$) (Table 4). The environmental variables responded differently to the treatments (Table 4): chlorophyll *a* was responsible for 61% of the explained variation, with TKN and SOM explaining less than 30% each (partial $R^2 = 0.28$ and 0.22, respectively). Standardized coefficients show strong but opposite effects on chlorophyll *a* (positive $\beta = 1.36$) and TKN (negative $\beta = 1.8$) variation, with no effect on SOM ($\beta = 1.01$).

The MANOVA was followed by *a priori* paired contrasts testing for differences in effects of particular treatments (Table 5). Within the high elevation, all three canonical (multivariate) tests comparing large snails with any combination including controls are highly significant ($p < 0.01$), the univariate contrasts show significant effects on chlorophyll ($p < 0.05$) and TKN, and there may be a significant effect on SOM (2 tests, $p < 0.07$). When comparing effects of tidal elevation, the controls did not differ in any test, but the multivariate (all treatments) test was highly significant. Large snails also had highly significant effects with tidal elevation overall ($p < 0.01$) and on chlorophyll ($p < 0.01$), and weaker effects on TKN and SOM (both $p < 0.05$). In the only contrasts of infected (large) and uninfected (small) snail effects, at the high elevation, the multivariate test was significant ($p < 0.05$) but none of the individual factors were significant (all $p > 0.16$). Also at the high elevation, small snails and the control had similar effects (all $p > 0.12$).

Overall, tidal elevation strongly affected multivariate (=canonical) and chlorophyll *a* correlations, and these effects are largely due to the large snails, which had highly significant effects overall and on the individual variables, especially chlorophyll *a*. Since there was no difference between the controls at the two tidal elevations, snails are the primary driver of differences, rather than any conditions associated with the different durations of emergence and submergence or other environmental variables.

While the lack of significance across all tests for small snails versus controls implies no difference between the treatments, the significant multivariate test for

large versus small snails suggests that small snails do have a weak effect on their environment. The high environmental variability (e.g. coefficients of variation in Table 3) suggests that the lack of significant individual effects for small snails reflects type II errors, and that they may exert some biologically important effects, despite the test results.

2.5 Discussion

By using snail size (>20 mm) as a proxy for infection of the introduced mud snail, *Batillaria attramentaria* by the parasitic trematode, *Cercaria batillariae,* we explored questions of A) whether *Batillaria* have measurable effects on their primary food (benthic diatoms) and on their habitat (sediment chemistry), and B) whether infected snails have greater impacts than uninfected snails. While loss of the small (uninfected) snail treatment at low tidal elevation limits direct comparisons of infected and uninfected snail effects to the high tidal elevation, we can compare the effects of infected snails at both elevations. Since smaller (uninfected) snails typically occupy only mid to high elevations, and since small snails placed lower in the intertidal soon move to higher levels (Chapter 1), a comparison of infected and uninfected snail effects at low elevation would have had limited ecological applicability in the field.

We first tested the hypothesis that feeding by the snails would reduce the density of diatoms compared with control cages without snails. Diatom size

frequency distributions are summarized in Figure 2. The total counts suggest large snails had different effects at the two tidal levels: diatom abundances were about 50% lower in large snail cages than in controls at low elevation. At high elevation, we were surprised that small, uninfected snails reduced diatoms more than large, infected. This suggests that small, uninfected snails could be more affected by food limitation than large, infected snails, a possibility that is supported by our observations of small snail dispersal (a proxy for food limitation) and the loss of the small snail/low elevation treatment. This could be especially true for snails at Kirby Park, where 73% of snails of mature size (> 20 mm long) are infected (Fabian, unpublished data). Uninfected snails at sites with high infection rates mature earlier (*C. californica*, Lafferty 2003a) and allocate more energy to reproduction (*C. californica*, Hechinger 2010), which could drive food-limitation for sexually mature, uninfected snails. Cages with small snails (high elevation only) did not differ from controls. While all size distributions in Figure 2 are highly skewed towards smaller size classes, those in both large snail treatments virtually lack a second mode of larger diatoms visible in the other three treatments.

The GLM analysis confirms that differences in both abundance and size distributions are real (Figure 3). The two large (infected) snail treatments are fitted with almost identical models that are distinguished from the other three by having significantly fewer diatoms overall and, especially, almost no large diatoms. We interpret this pattern to mean that feeding rates of infected (large) snails exceed the growth rates of diatom populations, and that they feed preferentially on larger

diatoms. It is also possible that the larger diatoms may be different species than the smaller ones.

Models for the two controls and the small snail treatment at high elevation form another group with higher overall abundances and many more large diatoms (Figure 3). Because the small snail treatment does not differ from the controls, this suggests that feeding rates of small snails are substantially lower than the diatom population growth rates. There is also no indication that small snails feed preferentially on particular size classes (or species) of diatoms.

The near complete elimination of the largest size classes of diatoms (>300 μm) by large snails also has implications for sediment stability. Since large, motile diatoms supply most of the extracellular polymeric substances (EPS) secreted by the microphytobenthos (MPB) (Miller et al. 1996), grazing by high densities of infected (large) snails may increase sediment erodibility and/or resuspension by selectively removing large diatoms. This has been demonstrated in other species. The snail *Hydrobia ulvae* increases erosion of sediments in field and laboratory settings by reducing diatom numbers and packaging surface sediments into fecal pellets, both of which reduce sediment cohesiveness (Andersen 2001, Andersen et al. 2002). The ecologically similar *C. californica* also has been reported to decrease sediment stability (Boyer and Fong 2005).

At high elevation, the smallest diatoms (< 50μm) increased when the largest diatoms were removed. Large diatoms are particularly important food sources for copepods and small fish, contributing to the efficiency of energy transfer to higher

trophic levels (Finkel at al. 2009). Shifts from larger to smaller diatoms could alter competitive interactions among the many taxa of macrofauna and meiofauna that consume diatoms. At low tidal elevation, small diatoms did not become more abundant when large diatoms were removed, so snails' effects on competitive interactions are likely to be stronger at high elevation. However, large snails decreased diatom abundances across size classes at low elevation, so infected snails might decrease the availability of food resources for infauna lower in the intertidal.

Snail effects on benthic chlorophyll a

Based on findings that *Batillaria* increased chlorophyll *a* in intertidal sediments in San Francisco Bay (Weiskel et al. 2007), we hypothesized that would also increase benthic chlorophyll *a* in Elkhorn Slough. In our experiment, only the large snails affected significantly chlorophyll *a* concentrations (Figure 4). At high elevation, chlorophyll *a* concentrations in large snail cages were approximately twice those in the controls and small snail treatments, even though there were fewer and smaller diatoms in large snail cages. Conversely, chlorophyll *a* concentrations in large snail cages at low elevation were much lower than the controls. These patterns suggest that snails may be altering competitive dynamics among diatoms and cyanobacteria. Since large snails doubled chlorophyll *a* concentrations relative to small snail and control treatments, their removal of large diatoms likely led to the proliferation of both the smallest (< 50 μm) diatoms and cyanobacteria, increasing MPB standing stock. MPB biomass measured as chlorophyll *a* is highly correlated

with sediment erodibility in some studies (Reïthmuller et al. 1998, Austen et al. 1999), but only weakly correlated in others. Weak correlations have been attributed to the importance of pelletization by snails and MPB assemblage composition (Andersen 2001, Reïthmuller et al. 2000).

In addition to removing the largest diatoms, large snails may have altered MPB biomass (chlorophyll *a*) and species assemblages by fertilizing small diatoms and cyanobacteria via direct inputs. It is also possible that disturbance (e.g., burrowing) by snails may stimulate efflux of nitrogen from sediments, but this seems unlikely in our experiment, since sediment TKN was higher (though not significantly) in control treatments. Snail burrowing can also disturb infauna that also consume the MPB (Dewar et al. 2008, Kelaher et al. 2003, Hamilton et al. 2003), and such interactions may have contributed to the effects of snails on diatoms and the MPB in high elevation treatments.

Snails had a very different effect on chlorophyll at the low tidal elevation, where large snails reduced chlorophyll *a* concentrations to ~25% of levels in the controls. Large snails' simultaneous (but non-significant) reduction of diatoms and (marginally significant) large reduction of chlorophyll *a* suggest that snails are depleting the MPB standing stock at low tidal elevation. Large snails did not become observably food-limited, however; we documented few large snail escapees from the low elevation cages (<10 total throughout the 10 week experiment). This contrasts with a study in San Francisco Bay, where *Batillaria* increased chlorophyll at both elevations (Weiskel et al. 2007). At low elevations, large snails probably reduce MPB

by grazing, and burrowing may further limit microphytobenthic production by increasing turbidity and hence limiting light availability to cells (e.g., Connor 1980, Connor and Edgar 1982). Lower standing stocks of MPB under reduced light conditions has been suggested as a cause of reduced sediment stability at lower elevations on intertidal mud flats (Austen et al. 1999).

Ecological implications

The differing effects of small (uninfected) and large (infected) snails on their food resources, and the variability in these effects at high and low tidal elevations underscores the context-dependency of species interactions, particularly in dynamic habitats with patchy resources. In our study, snails affected the abundance and size distributions of diatoms, the biomass of the MPB (using chlorophyll as a proxy), and very possibly the species composition of the MPB, all of which have implications not only for sediment stability but also for food web structure and functioning. Effects of snails on their resources are complicated because their effects on the MPB also involve indirect effects from such snail activities as burrowing, and from the snails' interactions with other benthic species. While removal of large diatoms and pelletization probably decrease sediment stability, increased MPB biomass (e.g. due to fertilization by snail feces) may compensate for these effects. Developing metrics for EPS contributions of diatoms and cyanobacteria by size and/or taxon would enhance understanding effects of changing MPB assemblages on

sediment stability, given that EPS production varies with growth rate and environmental conditions (Smith and Underwood 2000).

Because the dominant diatom size class can determine an assemblage's ecological function (Sugie and Suzuki 2015), any differential effects of small, uninfected and large, infected snails on diatom size distributions are likely to have cascading effects for other species. The MPB is an important food source that provides up to half of total estuarine production, and is in important determinant of benthic faunal assemblages (Compton et al. 2013). It is also clear that effects of *Batillaria* on mudflats involve complex interactions between their primary food resource and sediment chemistry. Understanding the roles that dominant snail grazers play in shaping the MPB assemblage and biomass, and how this may vary in space and time, is an important consideration in forward-thinking management of these systems.

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2.7 Figures and Tables

Figure 1: Diatom size distributions

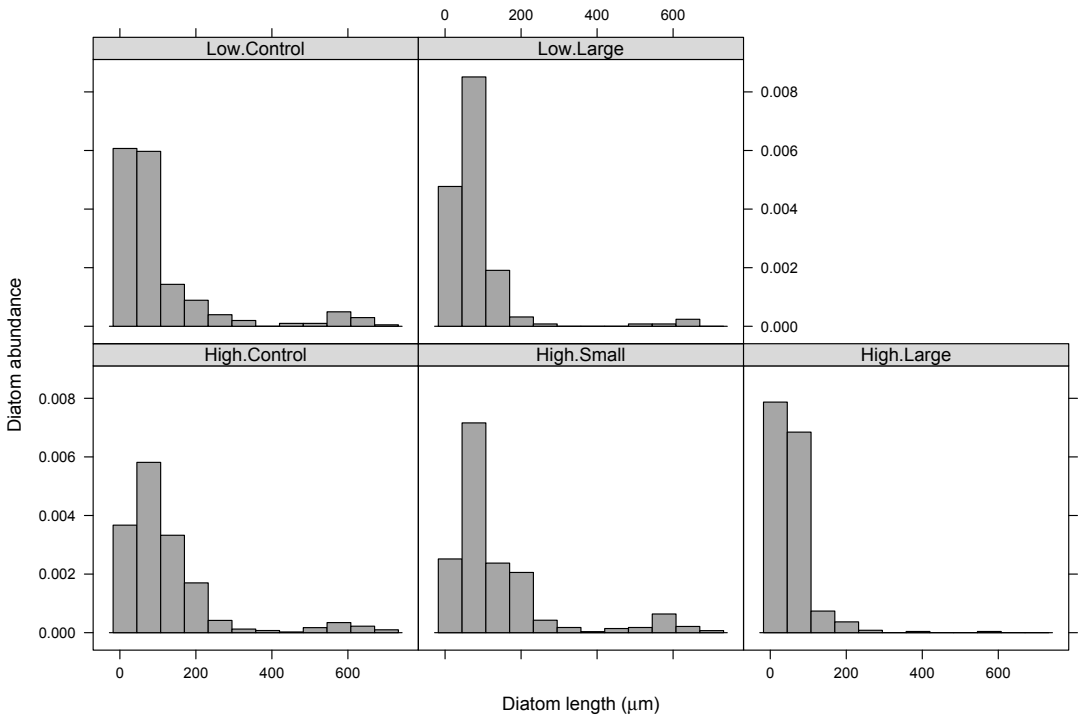

Figure 1: Size distributions of diatoms that were alive at the time of collection. Data are proportions of total counts from 2 slides in each cage.

Figure 2: GLM Results, Diatom size frequencies

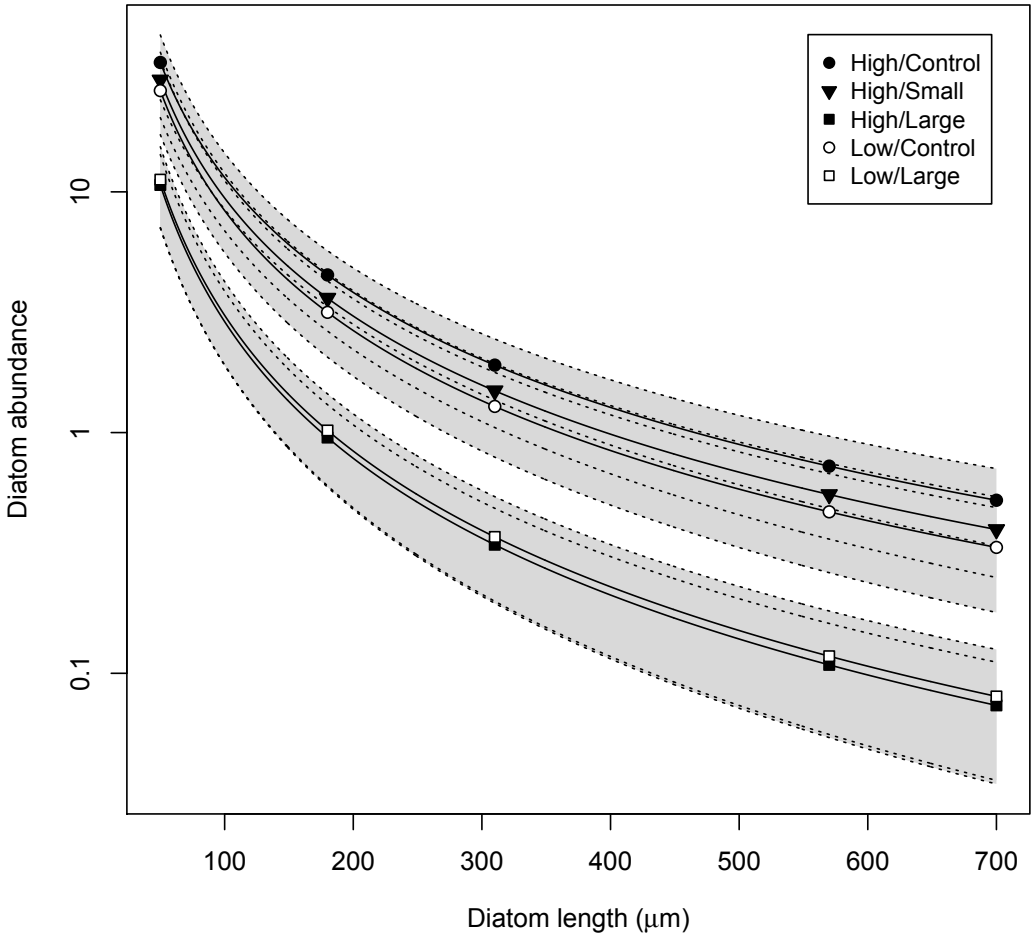

Figure 2. Negative binomial GLM where predicted abundance (μ) is regressed on the natural log of *50* μm length bin for each treatment, with a single shared intercept. Solid lines are predicted values for the indicated treatment according to respective symbol. Shaded regions about lines are 95% confidence regions between boundaries (dotted lines); symbols on lines are only for identification of treatments.

Figure 3: Benthic chlorophyll *a*

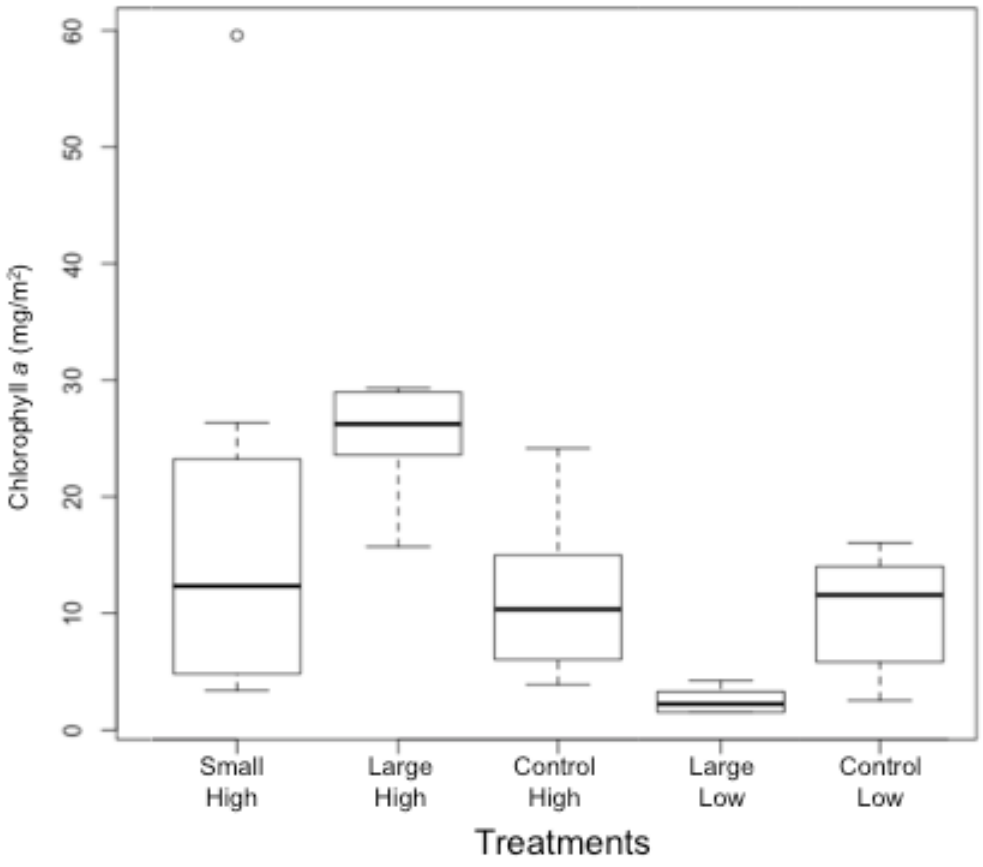

Figure 3: Benthic chlorophyll *a* in experimental cages. Boxplots show 25th, 50th, and 75th quartiles, and whiskers indicate 5th and 95th percentiles. The plot includes an extreme value in the small snail/high elevation treatment.

Figure 4: Sediment nitrogen content (TKN)

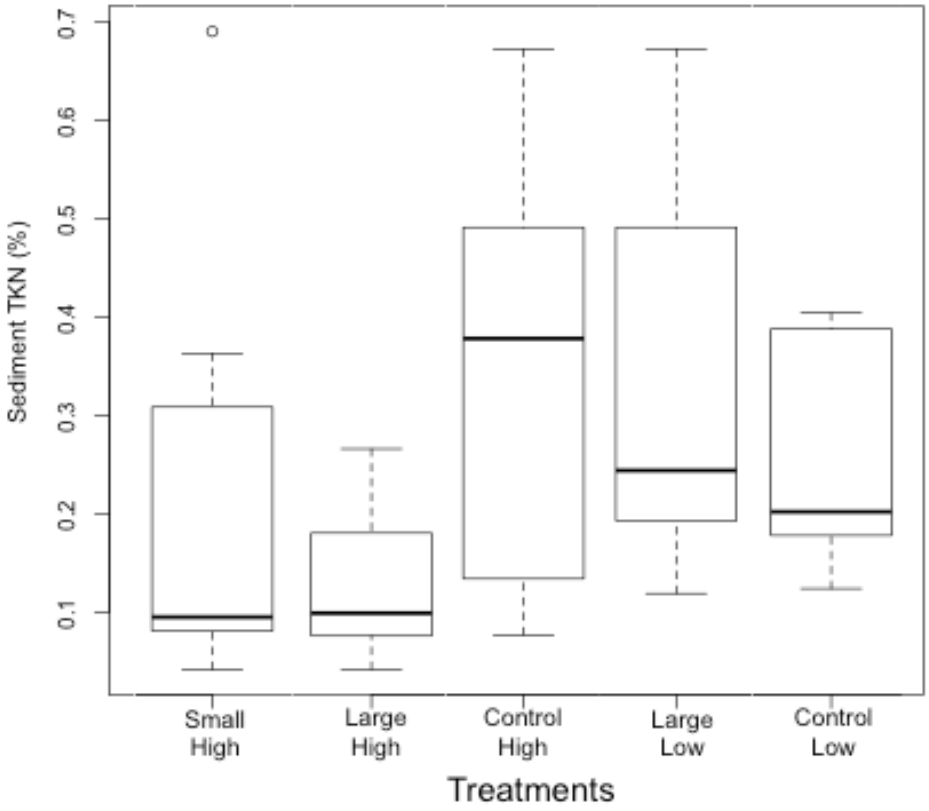

Figure 4: Sediment TKN in experimental cages. Boxplots show 25th, 50th, and 75th quartiles, and whiskers indicate 5th and 95th percentiles. The plot includes an extreme value in the small snail/high elevation treatment. Differences among treatments were not statistically significant.

Figure 5: Sediment organic matter

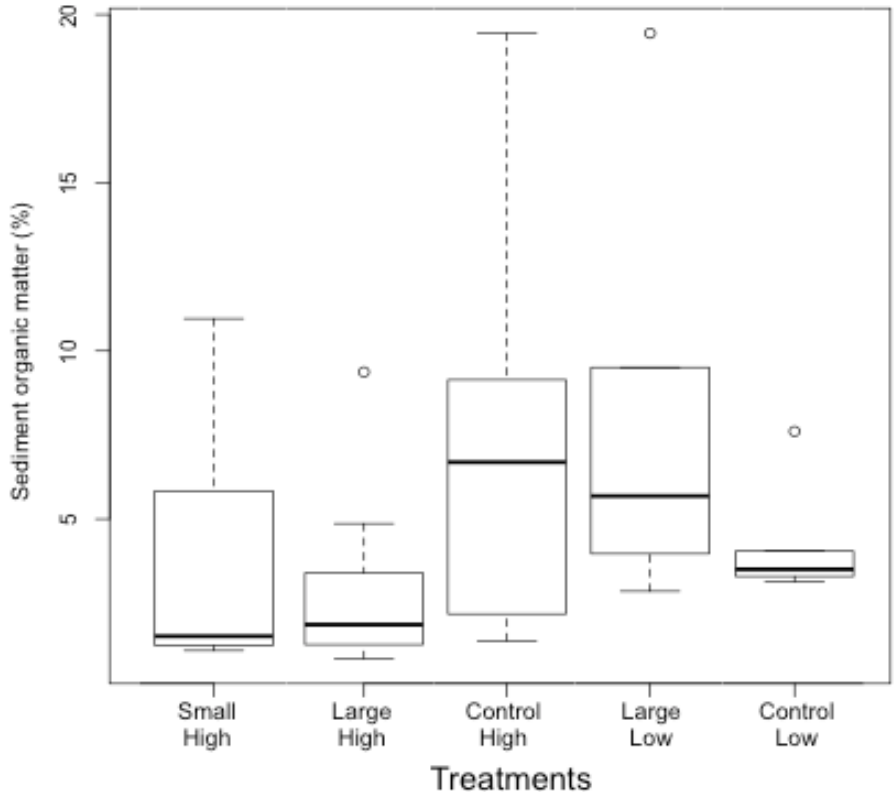

Figure 5: Sediment organic matter in experimental cages. Boxplots show 25th, 50th, and 75th quartiles, and whiskers indicate 5th and 95th percentiles. Some extreme values are included. Differences among treatments were not statistically significant.

Table 1: Diatom abundances and sizes

Treatment		Diatom abundance			Diatom length (μm)		
Snail	**Elevation**	**N**	**Mean**	**CI**	**N**	**Mean**	**CI**
Control	High	7	92	(67, 129)	649	253	(203, 316)
Control	Low	5	65	(44, 96)	324	272	(209, 354)
Small	High	8	56	(41, 77)	451	269	(219, 331)
Large	High	7	56	(40, 78)	390	134	(100, 180)
Large	Low	6	34	(23, 49)	201	153	(108, 216)

Table 1. Negative binomial means and 95% confidence intervals (CI) for pennate diatom abundance and lengths (dispersion 5.24 and 1.41, respectively). Abundance data for each cage were the sums of live diatoms on two slides; N (abundance) is the treatment replication (number of cages). Lengths were measured for each diatom counted; N (length) is the sum of all diatoms counted in all cages in that treatment.

Table 2: Slopes and confidence intervals for diatom size distributions

Model: $\ln(\mu) = \alpha + \ln Length * Treatment$

Parameter	Estimate	SE	z	p	Group
Intercept	9.75	0.400	24.36	< 0.001	NA
Control/High	-1.59	0.076	-20.75	< 0.001	A
Control/Low	-1.66	0.081	-20.54	< 0.001	A
Small/High	-1.63	0.077	-21.09	< 0.001	A
Large/High	-1.89	0.086	-22.03	< 0.001	B
Large/Low	-1.87	0.088	-21.41	< 0.001	B

Table 2. Negative binomial GLM where diatom abundance is regressed on the natural log of predicted values for 50μm bins for each treatment, with a single shared intercept (α). Parameter estimates for each treatment are slopes, and differences among treatments are indicated by group letters. μ is the predicted mean abundance. Deviance explained 62.5% of variance; dispersion = 0.662.

Table 3: Sediment properties

Snail	Elevation	N	Chl *a* (mg/m^2)	TKN (%)	SOM (%)
Control	High	7	9.52 ± 1.74 (0.48)	0.32 ± 0.08 (0.68)	6.76 ± 2.56 (1.00)
Control	Low	5	10.00 ± 2.53 (0.57)	0.26 ± 0.06 (0.49)	4.32 ± 1.93 (1.00)
Small	High	8	17.97 ± 6.65 (1.05)	0.21 ± 0.08 (1.04)	3.65 ± 1.29 (1.00)
Large	High	7	24.76 ± 1.75 (0.19)	0.13 ± 0.03 (0.67)	3.07 ± 1.16 (1.00)
Large	Low	6	2.51 ± 0.44 (0.43)	0.33 ± 0.09 (0.65)	7.86 ± 3.21 (1.00)

Table 3: Sediment properties (mean ± SE) after 10 weeks of exposure to experimental treatments, with coefficient of variation in parentheses. For large and control treatments at high tidal elevation, N = 6 for %TKN and %SOM due to the loss of one sample and elimination of an outlier from each.

Table 4: Sediment property MANOVA results

	Structural composition		Linear coefficients	
Variable	**Correlation**	**Partial R^2**	**Standardized**	**Raw**
Chl *a*	0.78	0.61	1.36	0.11
N	-0.53	0.28	-1.80	-9.47
SOM	-0.47	0.22	1.01	0.22

Table 4. First canonical correlations ($R^2 = 0.688$) for a MANOVA of the simultaneous effects of treatment on environmental variables. Wilks' Lambda = 0.312; $F_{12,74} = 3.43$; $p < 0.001$.

Table 5: Sediment property *a priori* contrasts

Snail treatments and Elevation

Group 1	Group 2	Canonical	Chl *a*	TKN	SOM
Lg at High	C at High	< 0.001***	0.010**	0.023**	0.071*
Lg at High	C+Sm at High	< 0.001***	0.021**	0.064*	0.212
Lg+Sm at High	C at High	0.002***	0.026**	0.034**	0.058*
All at High	All at Low	0.002***	0.003***	0.334	0.356
Lg at High	Lg at Low	< 0.001***	<0.001***	0.050*	0.050*
C at High	C at Low	0.711	0.817	0.417	0.284
Lg at High	Sm at High	0.043**	0.156	0.355	0.742
Sm at High	C at High	0.121	0.201	0.157	0.134

Table 5. Probabilities for multivariate *a priori* contrasts (testing equality of effects) between indicated combinations of treatments (group 1 versus group 2). The canonical p-values are Wilks' Lambda tests for multivariate significance. Values below each environmental variable are p-values for separate effects. Lg = large snails; Sm = small snails; C = control. High and Low = tidal elevations (* = p <0.10; ** = p <0.05; *** = p < 0.01).

Chapter 3: Responses of an opportunistic macroalga to an invasive mud snail

3.1 Abstract

Macroalgal blooms are common seasonal occurrences in estuaries that receive excess nitrogen from sources such as agriculture, waste treatment facilities, and urban runoff. Persistent algal blooms can cause many problems in estuaries, commonly driving shifts in water chemistry that can disrupt local ecological function. Bloom-forming macroalgae are sensitive to nitrogen limitation, and bloom persistence is primarily affected by nitrogen supply and grazing, in addition to other factors. Previous work has shown that dominant mud flat snails can facilitate the bloom-forming macroalga *Ulva* spp., either by increasing algal biomass or nitrogen content. We investigated the effects of the non-native Japanese mud snail, *Batillaria attramentaria,* on *Ulva lactuca*, in a 10 day laboratory experiment. We tested the hypotheses that snails would facilitate *Ulva* (as indicated by biomass, %N, C/N, and/or photosynthetic yield). We also hypothesized that snail effects would differ in low and high nitrate water, since failures to document effects of snails on *Ulva* in situ have been attributed to ample nitrogen availability. In our experiment, snails significantly increased algal biomass in high nitrate treatments, indicating that snails might exacerbate the growth of algal mats in nitrogen-replete water. In low nitrate treatments, snails significantly mitigated algal nitrogen loss, suggesting that snails could increase persistence of algal mats when nitrogen becomes scarce. Because the effects of snails in contact with algae and caged snails did not significantly differ, fertilization was likely the

mechanism by which snails affected the algae. Our results indicate that *Batillaria* can influence the growth and persistence of *Ulva* mats.

3.2 Introduction

More than half of US estuaries assessed by NOAA (Bricker et al. 2007) experience moderate to high levels of eutrophication, whereby excess nutrient inputs drive high primary productivity of phytoplankton and/or macroalgae, causing a host of negative effects. In estuaries, macroalgal blooms are often comprised of *Ulva* spp., an opportunistic green alga that responds to nutrient pulses with rapid colonization and growth (Dailer et al., 2012; Pederson and Borum 1997). Ulva's short life history allows it to outcompete other slow-growing algae when nutrients are high, especially in disturbed environments (Fletcher 1990). *Ulva* blooms can cause large changes in dissolved oxygen over diel periods, which can have deleterious effects on marsh plants and benthic invertebrates, driving a shift toward microbial loops and ecological decline (McGlathery 2001; Diaz and Rosenberg 1995; Baird et al. 2004). Both the causes and effects of macroalgal blooms are expected to intensify with intensified population and industrial activities as well as global change (Rabalais et al. 2009).

The primary factors affecting growth and persistence of marcoalgal mats include nutrient loading, grazing, and water residence times (Valiela et al. 1997). *Ulva* spp. need ample nitrogen, as well as co-limiting micronutrients such as iron, to sustain growth, and *Ulva* declines when nitrogen is scarce (Pederson and Borum 1997; Viaroli et al. 2005). Grazers may limit the growth and persistence of *Ulva* mats by reducing algal biomass; they can also stimulate growth via grazing of algal tissue or epiphytes, and fertilizing the algae. Accordingly, many studies have attempted to describe the effects of primary consumers on *Ulva*, such as isopods, amphipods (e.g.

Kamermans et al. 2002), polychaetes (e.g. Engelsen and Pihl 2008), and the mud snails *Ilyanassa obsoleta* (Gianotti & McGlathery 2001, Guidone et al. 2010, 2012, McLenaghan et al. 2011, Yarrington et al. 2012) and *Cerithidea californica* (Fong et al. 1997), as well as the grazing community as a whole (Geertz-Hansen et al. 1993). This study examines the effects of the non-native Japanese mud snail, *Batillaria attramentaria*, on *Ulva lactuca* in a central California estuary, Elkhorn Slough, where the snail is the dominant mud flat grazer. *Batillaria* is ecologically similar to *Ilyanassa* and *Cerithidea*, especially to *Cerithidea*, the native analog that is displaced where *Batillaria* is introduced (Byers 1999, 2000a, 2000b).

Previous work has generally shown positive effects of snails on *Ulva* spp. in the laboratory. Previous workers have documented snail facilitation of *Ulva* spp., measuring either increased algal biomass (Guidone 2010, 2012; McLenaghan et al. 2011; Yarrington et al. 2012) or nitrogen content (Fong et al. 1997; Gianotti and McGlathery 2001). Various mechanisms have been cited, including fertilization via snail excretions and burrowing-induced nitrogen efflux from sediments, and grazing of fouling microorganisms. (Guidone et al. 2010, Fong et al. 1997, Byers 1999); thus facilitative effects of snails on algae may have the potential to impact nutrient and food web dynamics (Fong et al. 1997). However, attempts to measure facilitation of *Ulva* by snails in field experiments have not yielded significant results; authors have attributed this to a lack of nutrient limitation in situ and an inability to exclude small grazers from experimental designs (Guidone 2012; Yarrington et al. 2013).

Study system

Batillaria attramentaria (= *B. cumingi, B. zonalis*), the Japanese mud snail, is a highly successful invader of intertidal habitats in estuaries from Boundary Bay, BC (Canada), to Elkhorn Slough, CA. Its native range is from Korea to Japan, where it is often the dominant invertebrate in salt marshes and on mudflats along the northeastern coast of Asia (Miura 2006b). *Batillaria* has displaced its native ecological analog, *Cerithidea,* in Elkhorn Slough, and has caused large declines in *Cerithidea* populations at sites where the snails co-occur. *Batillaria* is the dominant macrobenthic mud flat grazer in Elkhorn Slough.

Elkhorn Slough is a highly anthropogenically modified tidal wetland complex in central California that experiences moderate to hyper eutrophication. The Old Salinas River (OSR), the slough's primary freshwater source, carries highly nitrogen-enriched agricultural runoff, often exceeding 1000 μM NO_3^-; this water is diluted by Monterey Bay tidewaters (~20 μM NO_3^-) that pump it into the slough. Nitrate levels vary spatiotemporally in the slough, with monthly samples at 18 sites ranging from 0.00 mg/l to 56.40 mg/l NO_3^- (~910 μM) in July 2008- June 2009 (mean 1.74 mg/l, ~ 28 μM) (Hughes et al. 2010, 2011). In Elkhorn Slough, blooms characterized by *Ulva* spp. occur during late spring and summer months. In some areas, intertidal, subtidal, and floating algal mats can reach 90-100% coverage, driving hypoxia, particularly at sites behind tidal control structures (Hughes et al. 2010). *U. lactuca* and *U. intestinalis* are the most common species within intertidal algal mats in Elkhorn

Slough (Hughes et al. 2011); *Batillaria* can be found underneath, atop, and within mats (Figure 1).

Snails can impact *Ulva* mats directly by grazing the macroalgae and/or fouling microorganisms, and fertilizing algae via excreta or by burrowing, promoting nutrient exchange between the water column, microalgae, and sediment pools (Yarrington et al. 2012). These effects are likely most pronounced in tidally restricted areas during the dry season, corresponding with seasonality of *Batillaria* activity. *Batillaria* occupy mud flats in high densities (> 20,000 indiv/m^2 in some areas; Fabian, unpublished data) at many sites that experience severe macroalgal blooms during summer months. Because *Batillaria* are so numerous, even if per capita effects are small, the snails are likely to influence nitrogen cycling among sediment, water column, microalgal, and possibly macroalgal pools, especially when ambient nitrogen is low and water residence time is high.

No published work has been done to date on *Batillaria*'s interactions with macroalgae. *Batillaria* has been found to have varying effects on benthic microalgae in different estuaries (Bodega Bay, Dewar et al. 2008; Padilla Bay, WA, O'Connor et al. 2001; San Francisco Bay, Weiskel et al. 2007), ranging from increases to large decreases, suggesting that *Batillaria* may have varying effects depending on physicochemical and community contexts.

Based on the published work with *Ilyanassa* and *Cerithidea*, we hypothesized that *Batillaria* would facilitate *Ulva* spp., as indicated by algal responses (growth, %N, C/N and/or photosynthetic yield). To investigate this hypothesis, we examined

algal responses to contact with snails (allowing for grazing of algae or fouling microorganisms as well as nutrient inputs) and to snails separated from algae in cages (nutrient inputs only), in laboratory microcosms containing snails and algae in artificial seawater. We further hypothesized that magnitude of algal response would be a function of ambient nitrogen concentration, as indicated by previous workers' results. We used a factorial design, with three snail treatments at two nitrate levels, low and high.

3.3 Methods

Experimental design and set-up

The effects of snails on algae in different nutrient conditions were explored in a 3x2 factorial experiment. The three snail treatments were: snail contact (both grazing on algae and/or fouling organisms and fertilization possible); caged snails (only fertilization possible); and control (without snails). The two water nutrient treatments were low (~5 μM NO_3^-) and high (~50 μM NO_3^-), both well within the range of nitrate concentrations commonly occurring in Elkhorn Slough.

Each replicate contained a piece of algae and a cage (3 cm long, 3.25 cm dia PVC tube closed with 1 mm mesh on both ends), with or without snails, and a food pellet made of reconstituted algae. We employed 10 replicates of each treatment for a total of 60 replicates. The experiment ran for 10 days in a water table (190 x 60 x 15 cm) at Long Marine Laboratory, lit by two Philips 400W High Intensity bulbs (~250-300 μmol photons/m^2/s in the water table) with slowly flowing seawater (~13-14°C)

in a room with air temperature >23°C. Temperature in the water table was kept between 18-20°C for most of the experiment using four 75W Eheim submersible aquarium heaters. *Batillaria* tend to be more active at ~18-20°C than the incoming seawater temperature. On days 3 and 5, water temperature rose to 21.5°C, so the aquarium heaters and seawater flow were adjusted to return the water to the experimental temperature. Water temperature was measured twice daily (night time measurements were made on two days) at various positions within the water table. Submersible aquarium pumps were used to keep the water temperature uniform within the table, temperature varied by ~1°C due to proximity of heaters and incoming seawater and slightly varying light conditions within the table. The positions of replicates were randomized daily to control for variation in temperature and light conditions within the table.

Experimental microcosms (300 mL glass culture dishes with Petri dish tops) were filled with 300mL of artificial seawater (Salinity ~ 33; measured with a hydrometer) of low or high nitrate concentration. Snail treatments contained algae and 4 snails (18-20 mm long), either outside or inside the cage, and control replicates contained only algae and cages. Snails were scrubbed with a stainless steel brush and starved in artificial seawater for 24 hr before being added to microcosms; they were introduced 24 hours before the experiment began. To test for effects of snail fertilization, it was necessary for all snails to produce feces. Therefore, the caged snails were given food pellets (comprised of ~1 cm^3 freeze-dried and ground *Ulva* mixed with Bacto Agar for a tissue/water ratio similar to living *Ulva* tissue, as

detailed in Thornber et al. (2008)). Food pellets were included in all cages across treatments. Water and food pellets were replaced once every 2 days throughout the experiment. The experiment began at ~3:30 am on Day 1; water was changed in experimental chambers at ~18:00 on Days 2, 4, 6, and 8. Water samples from microcosms were collected on Day 6 when water was replaced in the chambers, and on Day 10 when the experiment ended; water had been in microcosms for ~48 h when it was collected.

Artificial seawater (Coralife Scientific Grade Marine Salt) containing no nitrates or phosphates was prepared in 70 L plastic trash cans to S ~ 33 using deionized water. Concentrated NO_3^- solution from an algal culturing kit (Bigelow Marine Laboratory, East Boothbay, ME) was added to the artificial seawater using a digital micropipet to reach target levels of 5 μM NO_3^- for low nitrate water and 50 μM NO_3^- for high nitrate water. Five batches of water were mixed throughout the algal acclimation period; the first four batches were used to acclimate the algae, and all water used during the experimental period came from the final (fifth) batch. Actual nitrate concentrations of batches varied from target nitrate concentrations; low nitrate water ranged from 5.52-8.04 μM NOx; high nitrate water ranged 49.95-56.9 μM NOx. Low nitrate water used in experimental microcosms was 8.04 μM NO_x; high nitrate experimental water was 51.3 μM NOx. Artificial seawater was aerated with submersible air pumps (Fluval Sea brand, 425 GPH) and covered with black plastic sheeting secured with duct tape to prevent evaporation and contamination of the water until used.

Water samples were taken from all microcosms on days 5 and 10 of the experiment and frozen for subsequent nutrient analysis (NO_3^-, NH_4^+, PO_4^{3-}, Lachat QuikChem 8000 Flow Injection Analyzer) at the Marine Analytical Laboratory at UCSC.

Experimental algae

Ulva lactuca was collected from Monterey Bay, since at the time of the experiment (May 2014), sufficient *Ulva* was not available in Elkhorn Slough. Algae were held in a water table (described above) with flowing seawater (~13-14°C) in the laboratory for one week before acclimation to artificial seawater, so as to minimize stress to algae associated with collection. Experimental algae were rinsed in fresh water, then prepared for acclimation by rubbing with towels to remove as many fouling microorganisms (bacteria, epiphytic microalgae) as possible. Algae were acclimated to low or high nitrate artificial seawater in large storage containers (75 x 40 x 15 cm) equipped with submersed water pumps (Fluval Sea brand, 425 GPH). The containers were kept in the phytoplankton lab at Long Marine Lab, under controlled light conditions (fluorescent 32W bulbs, ~200 μmol photons/m^2/s). These lighting conditions differ from the experimental conditions, but this was necessary due to spatial constraints. Artificial seawater was changed every other day during the 7 day acclimation period. To minimize epiphytic diatom growth, algae were scraped with towels during water changes. Algae with the least epiphytes were selected for experimental microcosms.

Prior to the experiment, algae were rinsed in fresh water, rubbed with towels, and hand-torn into pieces (in pilot experiments, this caused less damage than cutting with scissors or other implements) of similar size, dried in a salad spinner for 20 spins, and finally weighed before being put into microcosms. Initial algal mass ranged from 0.62 to 1.18 g (mean initial algal weight was 0.84 g). Because the algal sheets varied in size, some supplied algae for several (≤ 10) microcosms, while others supplied only 2-3. Algae pieces were assigned randomly to microcosms within the proper nitrate treatment. Photosynthetic yields (capture of photosynthetically-active radiation) of the algal pieces were measured using a Walz Teaching PAM; three measurements were made on each piece of algae before being randomly assigned to experimental microcosms. At the end of the experiment, three more measurements of photosynthetic yield were taken and all remaining algae were rinsed, dried, and weighed. Algae were then freeze-dried, ground with a mortar and pestle, and prepared for elemental analysis (Carlo Erba 1108 elemental analyzer interfaced with ThermoFinnegan Delta Plus XP isotope ratio mass spectrometer) at UCSC's Stable Isotope Lab.

Statistical Analyses

Factorial analyses of variance (ANOVA) were performed on the changes to each algal metric (mass, %N, C/N, yield. One-way ANOVAs of algal metrics were run for each nitrate treatment in order to assess whether effects of snails were different at low and high nitrate. Interactions among factors were tested for in all

analyses, and no significant interactions were found, so they were not included in analyses.

To assess whether snail or nitrate treatment influenced nutrient concentrations algal drawdown of nutrients in chambers, factorial ANCOVAs were performed for [NOx], [PO_4^{3-}], and [NH_4^+] in experimental water on Days 6 and 10. Corresponding nutrient measurements (e.g. [PO_4^{3-}], and [NH_4^+] as covariates for analysis of [NOx]) and initial algal mass were included as covariates for Day 6 analyses. Day 10 analyses used the same covariates, but included final mass rather than initial algal mass as covariates. Statistical analyses were performed in JMP Pro 12.0.1.

3.4 Results

Factorial analyses indicated that snails significantly affected changes to algal %N and C/N, while nitrate treatment did not significantly affect algal %N. For some response variables (algal mass, %N, C/N), effects of snail treatment differed in low and high nitrate treatments. When snail treatment was significant, post-hoc Tukey's HSD indicated that contact and caged snail treatments did not differ significantly in their effects on algae. Data tables and figures summarizing algal responses are included in Appendix I; tables summarizing water chemistry data are included in Appendix II.

Algal Responses

Algal mass

Algae grew larger in all treatments, with the greatest increases in caged snail treatments (Figure 1). In the factorial ANOVA (Table 1), snail treatment was marginally significant ($p = 0.0714$) and nitrate treatment was not significant ($p = 0.8351$). In separate one-way ANOVAs (Table 2), caged snails significantly increased algal mass in high nitrate ($p = 0.0300$) but not low nitrate water ($p = 0.2776$). Tukey's HSD for the high nitrate treatment showed that algae with caged snails grew more than algae in control treatments, but algae in the snail contact treatment did not differ significantly from either caged or control treatments.

Algal nitrogen content

Algal nitrogen content (%N) declined in all treatments except the high nitrate/snail contact treatment (Figure 2). In the factorial ANOVA (Table 3), snail treatment, but not nitrate treatment had significant effects on algal %N ($p = 0.0058$ and 0.1120, respectively). Tukey's HSD indicated that changes to algal %N differed significantly in the snail contact and control treatments; changes to algal %N in the caged snails treatments were intermediate and did not differ from snail contact or control treatments. Separate one-way ANOVAs (Table 2) for low and high nitrate treatments indicated that impact of snails differed between nitrate treatments. In low nitrate, snail treatment had significant effects on changes to algal nitrogen content (p

= 0.0314), but snail treatment did not significantly affect changes to algal nitrogen content in high nitrate ($p = 0.1271$). In low nitrate, snail contact differed significantly from the control treatment, while caged treatment did not differ from either contact or control treatments.

Algal C/N

Algal C/N increased in all treatments, with the lowest increase in the high nitrate/snail contact treatment and the greatest increase in the low nitrate/control treatment (Figure 3). In the factorial analysis (Table 4), snail treatment significantly affected changes to algal C/N ($p = 0.0004$); nitrate treatment was not significant. Snail contact and caged snail treatments differed significantly from the control treatments, but not from one another. Accordingly, in both low and high nitrate treatments, snail treatment significantly affected changes to algal C/N ($p = 0.0282$ and 0.0169, respectively, Table 5). Snail contact and control treatments differed significantly from one another, while the caged snail treatment was not significantly different from either treatment in both low and high nitrate (Tukey's HSD).

Algal photosynthetic yield

Algal photosynthetic yield decreased similarly across treatments (Figure 5). Nitrate treatment, but not snail treatments, significantly affected photosynthetic yield, with smaller decreases in high nitrate water (Tables 2,5).

Water chemistry

NO_x

Algae in all treatments drew down [NO_x] in experimental chambers to similar levels, with mean [NOx] in chambers increasing from Day 6 to Day 10 in all treatments except for the control/high nitrate treatment (Figure 5). On Day 6, mean [NOx] ranged from 0.79 ± 0.11 μM for the caged snail/low nitrate treatment to 1.65 ± 0.64 μM for the caged snail/high nitrate treatment. The caged snail/high nitrate treatment included an anomalous measurement of 6.71 μM NOx (~6x greater than the mean; this value was excluded from analyses); when this value was excluded, the mean decreased to 1.02 ± 0.12 μM, lower than the control (no snails)/high nitrate treatment (1.15 ± 0.06 μM NOx). On Day 10, mean [NOx] ranged from 1.14 ± 0.11 μM in the control/high nitrate treatment to 1.52 ± 0.15 μM in the caged snail/low nitrate treatments. Neither the Day 6 nor the Day 10 ANCOVA indicated significant effects of factors or covariates on [NOx] in experimental microcosms (Tables 6-7).

PO_4^{3-}

The artificial seawater mix was free of phosphate (batches ranged from -0.18 to -0.07 μM PO_4^{3-}); levels of [PO_4^{3-}] remained low in experimental chambers, with the greatest values observed in the caged snail treatments (1.07 ± 0.14 μM and 1.27 ± 0.15 μM on Days 6 and 10, respectively, in low nitrate; 0.89 ± 0.25 μM and 0.70 ± 0.13 μM on Days 6 and 10, respectively, in high nitrate)(Figure 6).

The Day 6 ANCOVA indicated that snail treatment significantly affected phosphate levels in experimental chambers (p = <0.0001, Table 7). Snails continued to significantly affect phosphate in experimental chambers on Day 10; [NH_4^+] was also a significant covariate (p = <0.001 and 0.0021, respectively, Table 7).

NH_4^+

[NH_4^+] did not show clear patterns among treatments (Figure 7). Mean [NH_4^+] in experimental chambers ranged from 0.40 ± 0.26 μM (no snails/high nitrate) to 1.63 ± 0.26 μM (snail contact/low nitrate) of Day 6 and from 0.36 ± 0.09 μM (no snails/high nitrate) to 5.83 ± 1.92 μM (caged snail/low nitrate) on Day 10. Snail treatment significantly affected measured [NH_4^+] in experimental chambers on Day 6 (p = 0.0002, Table 8), with water from snail contact treatments being significantly higher in [NH_4^+] than in caged or control treatments, but snail treatment was not significant on Day 10 (p = 0.3574). None of the covariates significantly affected [NH_4^+] in experimental chambers.

3.5 Discussion

Overall algal condition (as indicated by %N, C/N, photosynthetic yield) was lower at the end of the experiment, though algae gained biomass in all treatments. Snails increased algal biomass in high nitrate water, and they also affected algal

nitrogen content and C/N in the factorial analyses and particularly in low nitrate water. Negative changes to algal health parameters were greater in control treatments relative to snail contact or caged snail treatments.

Algal declines were likely due to experimental conditions not being ideal for *Ulva*, since macroalgae tends to perform better in flowing water. The use of artificial seawater could have contributed to algal stress, since it was free of phosphorus, an essential macronutrient. Algae could have also undergone thermal stress when temperatures rose in the water table. Nonetheless, algae gained biomass, and did not visibly senesce, as did some algae in pilot studies we conducted.

We hypothesized that if snails were both fertilizing and grazing *Ulva*, the algae would grow the most in caged snail treatments. This was true at both nitrate levels, suggesting that snails were both fertilizing and grazing on algae. Differences in algal growth were not different in snail contact and caged snail treatments, however, so snails primarily affected the algae by fertilizing it. Although snail treatment was not significant in the factorial ANOVA, caged snails significantly increased algal mass in high nitrate treatments. This suggests that snails might facilitate the build up of algal mats by promoting the growth of macroalgae during nutrient pulses.

Our results contrast with Guidone's (2010, 2012) findings that mud snails (*I. obsoleta*) increased algal growth, but not nitrogen content. Snails mitigated declines in algal nitrogen loss, significantly affecting changes to algal nitrogen content in the factorial ANOVA. In one-way ANOVAs, mitigation of algal nitrogen loss by snails

was significant in low nitrate treatments only. Algae in the snail contact treatment lost significantly less nitrogen than in control treatments with no snails, in which nitrogen loss was the greatest. The pattern of algae in snail contact treatments having the greatest positive changes in nitrogen content, with the most nitrogen loss in control treatments, is the same as we observed in pilot studies using natural seawater, but in those studies snails increased algal nitrogen content.

The loss of nitrogen across treatments (with the exception of a small increase in the snail contact/high nitrate treatment) might have been due to characteristics of the artificial seawater, particularly its lack of phosphate. Limitation by one macronutrient can inhibit macroalgal uptake of other macronutrients (Perini and Bracken 2014), so lack of phosphorus could have limited uptake or storage of nitrogen by the algae. The algae consistently drew down nitrate to similar levels (~1.5 μM [NOx]) in experimental jars. Perhaps lack of phosphorus inihibited nitrogen storage, or uptake at levels less than ~1.5 μM, since nitrogen and phosphorus interact in their effects on *Ulva* spp. at below-optimum concentrations (Steffensen 1976). *Ulva* spp. sometimes leak nitrogen as dissolved organic nitrogen (DON) during growth, resulting in nitrogen dilution (Fong et al. 2004, Boyer and Fong 2005, Perez-Mayorga et al. 2011). Algae might have taken up nitrate from experimental water and leaked it as DON as it gained biomass. Nitrogen storage and uptake is not always coupled to growth; and *Ulva* may delay growth in favor of nutrient uptake (Fong et al 2004).

Our finding that snails mitigated algal nitrogen loss is consistent with that of Fong et al. (1997) (for *C. californica*) and Gianotti and McGlathery's (2001) (for *I. obsoleta*) findings. Both of these designs included sediment, and water in experimental chambers was not changed, so it is difficult to compare our outcomes to theirs. Fong et al. (1997) attributed fertilization effects to burrowing-induced nitrogen efflux from the sediment layer to the microcosm water. Their design used estuarine water with a different nutrient profile (initially 4.14 μM NO_3^-, 9.84 μM NH_4^+, 55.02 μM TKN) than was used here, and the water was not changed over the 21 day experiment. Gianotti and McGlathery's (2001) attributed increases in algal nitrogen to fertilization by snails via excreta. They also used estuarine water that was not changed over the 24 days, and nutrient concentrations were not measured. This study did not include sediment from Elkhorn Slough, as it became highly anoxic in preliminary trials, making chambers inhospitable to either snails or algae.

Algal C/N increased for algae in all treatments, but snails significantly affected algal C/N in both low and high nitrate treatments. Algae in the snail contact treatments had significantly lower increases in C/N than algae in control treatments, and increases in algal C/N were intermediate in the caged snail treatments. The stronger effect of snail contact treatments than caged snails on algal nitrogen loss and C/N indicates that direct snail interaction with algae, in addition to fertilization, was important to minimizing increases to algal C/N. Snails could have been grazing tissue that was losing nitrogen, or clearing algae of fouling organisms that were inhibiting algal nitrogen storage. *Ulva lactuca* is chemically defended and has been found to

deter some herbivores (Erickson et al. 2006), so snails might have been more able to consume damaged algal tissue that was not defended, mitigating algal nitrogen loss and increases to C/N. By reducing increases to algal C/N, snails could improve the quality of *Ulva* as a food source for other consumers.

It is clear that snails can play an active role in cycling nitrogen among water column, algal, and sediment pools. Differences in algal biomass, nitrogen content, and C/N caused by snails were small in our experiment, but it is likely that *Batillaria* could enhance algal growth and/or nitrogen content in Elkhorn Slough, where the snails are abundant.

Conclusions

This study showed that *Batillaria attramentaria*, a non-native mud snail that dominates mud flats in Elkhorn Slough, can facilitate *Ulva* blooms that seasonally plague the slough. We found that the snails increased algal biomass in high nitrate water, and snails mitigated algal nitrogen loss and increases to C/N in low nitrate water. We also showed that changes to algal health metrics caused by snails are different in low and high nitrate water. Our findings suggest that *Batillaria* could intensify the development of algal mats when nitrogen is abundant, and that the snails could increase the persistence of algal mats when less nitrogen is available. Interestingly, we saw differences in algal responses to snails in low (8.04 μM) and high (50μM) nitrate treatments that did not represent extreme highs or lows relative to nitrate conditions within Elkhorn Slough. Future studies could explore snail effects

within and outside this range to further explicate algal responses to snails. Inclusion of sediments in future experimental designs would also be informative.

3.6 Works Cited

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3.7 Figures and Tables

Figure 1: *Batillaria* and *Ulva*

Figure 1: *Batillaria* at densities exceeding 10,000 individuals/m^2 with *Ulva* mat at Whistlestop Lagoon in Elkhorn Slough in January 2011.

Figure 2: Percent changes in algal mass

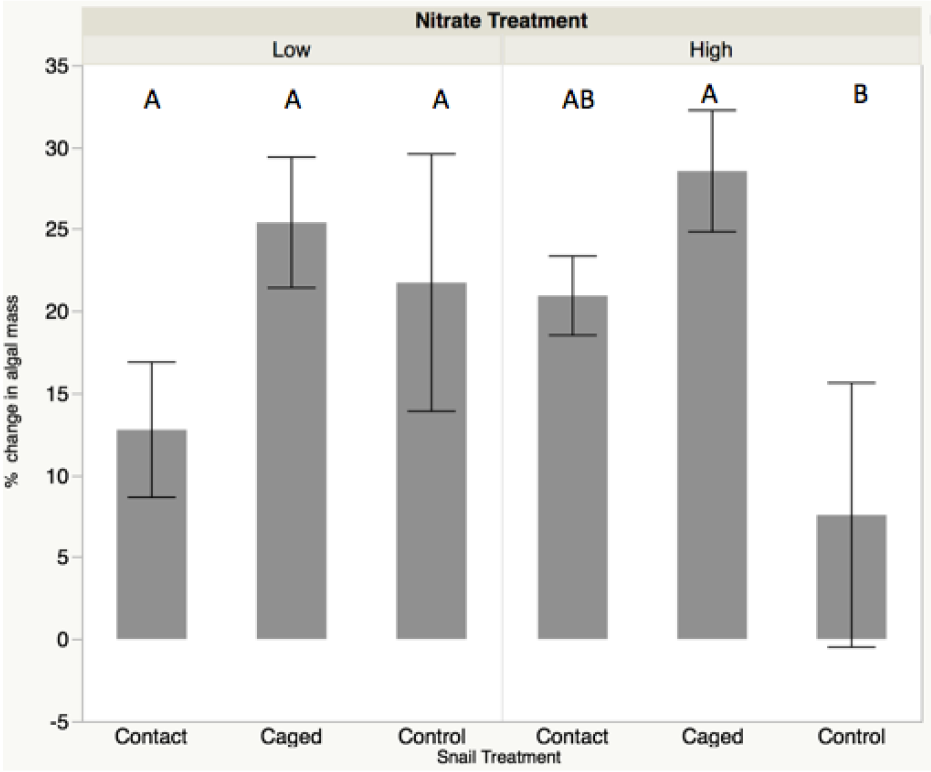


Figure 2: Mean percent change in mass of algae pieces over the course of the 10-day experiment, with standard error bars. The left panel shows percent changes in mass for the low nitrate treatment; the right panel shows percent changes in mass for the high nitrate treatment. Different letters above bars indicate statistically different changes in algal mass among snail treatments in separate one-way ANOVAs run to assess whether effects of snails differed in low and high nitrate treatments. For mean initial and final algal masses, see Appendix I, A.

Figure 3: Changes in algal nitrogen

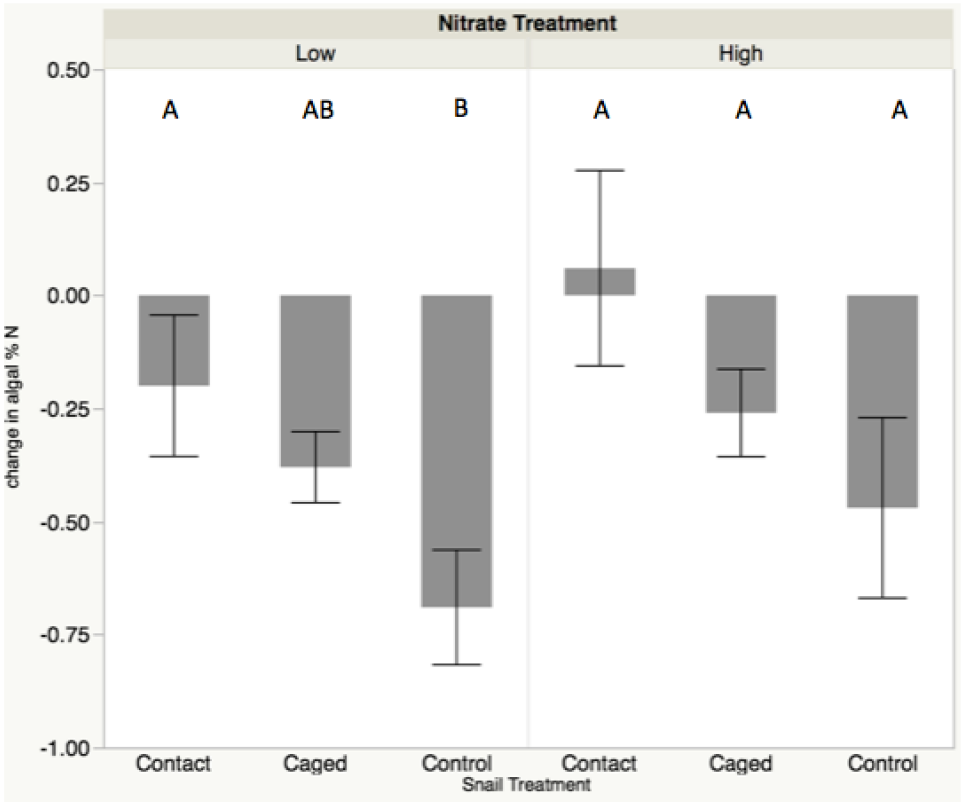


Figure 2: Mean change in algal N content (%N) of algae pieces over the course of the 10-day experiment, with standard error bars. The left panel shows changes in algal N for the low nitrate treatment; the right panel shows changes in algal N for the high nitrate treatment. Different letters below bars indicate significantly different changes in algal N content among snail treatments in separate one-way ANOVAs run to assess whether the effects of snails differed in low and high nitrate treatments. For mean initial and final algal N content, see Appendix I, B.

Figure 4: Changes in algal C/N

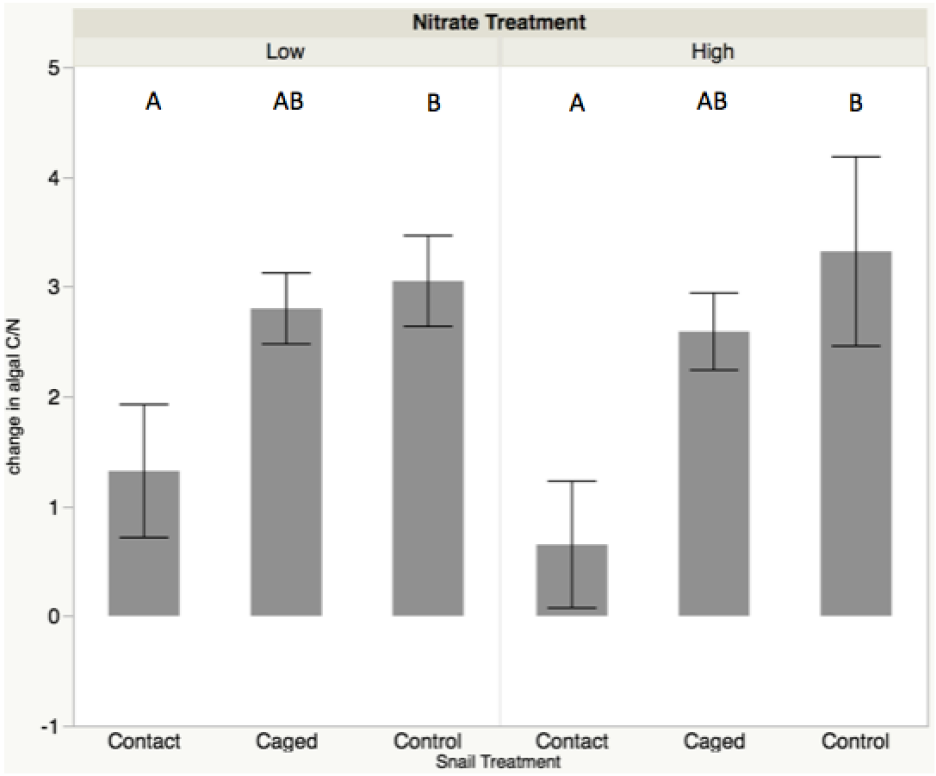


Figure 4: Mean change in C/N of algae over the course of the 10-day experiment, with standard error bars. The left panel shows changes in algal C/N for the low nitrate treatment; the right panel shows changes in algal C/N for the high nitrate treatment. Different letters above bars indicate significantly different changes in algal C/N among snail treatments in separate one-way ANOVAs run to assess whether snail and/or co-variate effects differed in low and high nitrate treatments. For mean initial and final algal C/N, see Appendix I, C.

Figure 5: Changes in algal photosynthetic yield

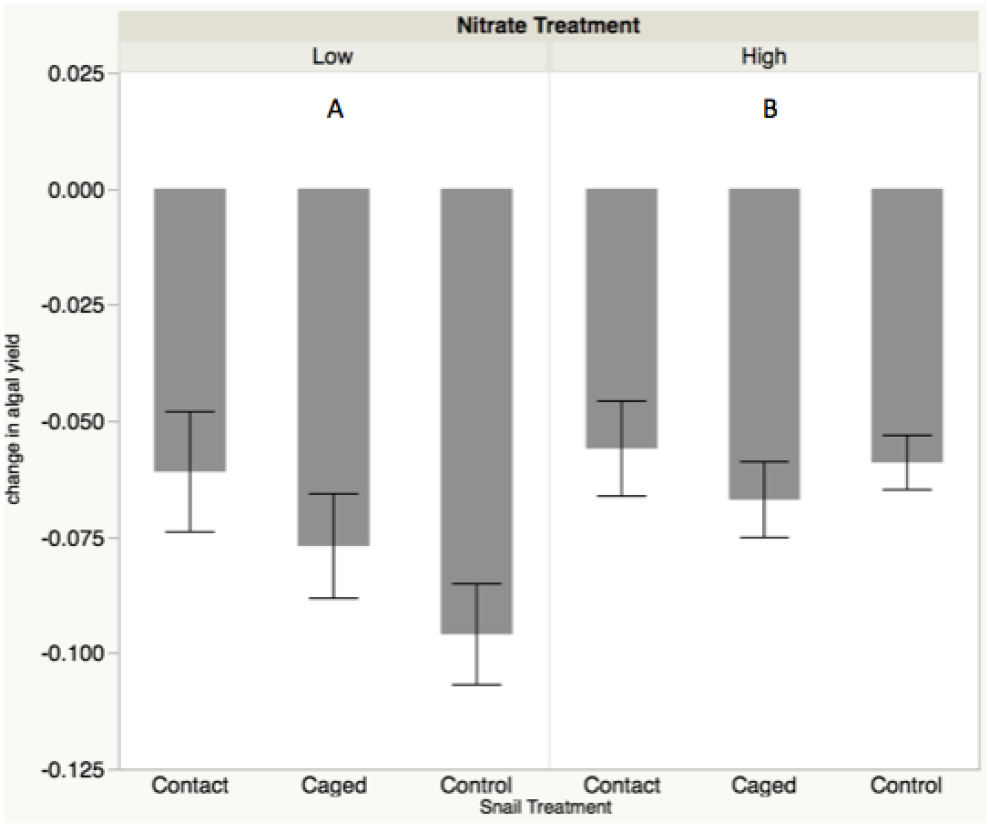


Figure 5: Mean change in photosynthetic yield of algae pieces over the course of the 10-day experiment, with standard error bars. The left panel shows changes in algal C/N for the low nitrate treatment; the right panel shows changes in algal C/N for the high nitrate treatment. In the factorial ANOVA, changes in photosynthetic yield were significantly different in low and high nitrate treatments, but not among snail treatments. For mean initial and final photosynthetic yield, see Appendix I, D.

Table 1: Factorial ANOVA Results, changes to algal mass

Effect Source	DF	SS	F-ratio	p
Snail Treatment	2	1726.04	2.77	0.0714
NO_3^- Treatment	1	13.63	0.04	0.8351

Table 1: Results of factorial ANOVA run using percent change in algal mass as the response variable, with snail and nitrate treatment as effect sources. Snail treatment was marginally significant.

Table 2: One-Way ANOVA Results, changes to algal health parameters

		Low NO_3^-			**High NO_3^-**		
Response	DF	SS	F-ratio	p	SS	F-ratio	p
Mass	2	845.17	1.34	0.2776	2254.15	4.00	0.0300*
%N	2	1.23	3.94	0.0314*	1.42	2.23	0.1271
C/N	2	17.49	4.08	0.0282*	38.08	4.77	0.0169*
Photosynthetic yield	2	0.01	2.23	0.1268	0.00	0.47	0.6295

Table 2: Results from separate one-way ANOVAs run for low and high nitrate treatments, using changes to algal metrics as response variables, with snail treatment as the effect source. Asterisks indicate statistically significant effects.

Table 3: Factorial ANOVA Results, changes to algal %N

Effect Source	DF	SS	F-ratio	p
Snail Treatment	2	2.60	5.65	0.0058*
NO_3^- Treatment	1	0.60	2.61	0.1120

Table 3: Results from factorial ANOVA run using change to algal %N as the response variable, with snail and nitrate treatment as effect sources. The asterisk indicates a statistically significant effect.

Table 4: Factorial ANOVA Results, Final Algal C/N

Effect Source	DF	SS	F-ratio	p
Snail Treatment	2	53.36	8.90	0.0004*
NO_3^- Treatment	1	0.62	0.21	0.6510

Table 4: Results from factorial ANCOVA run using change to algal C/N as the response variable, with snail and nitrate treatment as effect sources.

Table 5: Factorial ANOVA Results, change in algal photosynthetic yield

Effect Source	DF	SS	F-ratio	p
Snail Treatment	2	0.0038	1.83	0.1706
NO_3^- Treatment	1	0.0045	4.30	0.0426*

Table 5: Results from factorial ANOVA with change to photosynthetic yield as the response variable, and snail and nitrate treatment as effect sources. The asterisk indicates a statistically significant effect.

Table 6: Factorial ANCOVA Results, experimental chamber water [NOx] and [PO_4^{3-}], Day 6

			[NOx]			[PO_4^{3-}]	
Effect Source	DF	SS	F-ratio	p	SS	F-ratio	p
Snail Treatment	2	0.0381	0.14	0.8707	9.4328	31.75	<0.0001*
NO_3^- Treatment	1	0.2538	1.85	0.1802	0.0037	0.03	0.8742
[PO_4^{3-}] Day 6	1	0.0019	0.02	0.8899	NA	NA	NA
[NOx] Day 6	1	NA	NA	NA	0.0029	0.02	0.8890
[NH_4^+] Day 6	1	0.0066	0.07	0.7954	0.1000	0.67	0.4159
Initial algal mass	1	0.0933	0.96	0.3330	0.0015	0.01	0.9200

Table 6: Results from separate factorial ANCOVAs run for [NOx] and [PO_4^{3-}] on Day 6, run using snail and nitrate treatments as factors, and corresponding Day 6 nutrient concentration measurements, and initial algal mass, as covariates.

Table 7: Factorial ANCOVA Results, Experimental chamber water [NOx] and [PO_4^{3-}], Day 10

			[NOx]			[PO_4^{3-}]	
Effect Source	DF	SS	F-ratio	p	SS	F-ratio	p
Snail Treatment	2	0.0306	0.12	0.8800	8.2175	55.14	<0.0001*
NO_3^- Treatment	1	0.3468	2.70	0.1065	0.2166	2.91	0.0941
[NOx] Day 10	1	NA	NA	NA	0.0027	0.04	0.7591
[PO_4^{3-}] Day 10	1	0.0047	0.04	0.8498	NA	NA	NA
[NH_4^+] Day 10	1	0.0937	0.73	0.3974	0.7800	10.47	0.0021*
Final algal mass	1	0.0109	0.09	0.7717	0.1275	1.71	0.1964

Table 7: Results from separate factorial ANCOVAs run for [NOx] and [PO_4^{3-}] on Day 10, run using snail and nitrate treatments as factors, and the corresponding Day 10 nutrient concentration measurements and final algal mass, as covariates.

Table 8: Factorial ANCOVA Results, Experimental water [NH_4^+], Days 6 and 10

			Day 6			Day 10	
Effect Source	DF	SS	F-ratio	p	SS	F-ratio	p
Snail Treatment	2	20.7216	10.15	0.0002*	13.6750	1.05	0.3574
NO_3^- Treatment	1	0.1130	0.11	0.7409	7.4694	1.15	0.2892
[NOx] Day 6	1	0.0694	0.07	0.7954	NA	NA	NA
[PO_4^{3-}] Day 6	1	0.6877	0.67	0.4159	NA	NA	NA
[NH_4^+] Day 6	1	NA	NA	NA	NA	NA	NA
[NOx] Day 10	1	NA	NA	NA	4.7444	0.73	0.3974
[PO_4^{3-}] Day 10	1	NA	NA	NA	68.2091	10.47	0.0021*
Initial mass	1	0.0398	0.04	0.8444	NA	NA	NA
Final mass	1	NA	NA	NA	3.6205	0.56	0.4593

Table 8: Results from separate factorial ANCOVAs run for [NH_4^+] on Days 6 and 10, run using snail and nitrate treatments as factors. For Day 6, corresponding Day 6 nutrient concentration measurements and initial algal mass were included as co-ariates. For Day 10, corresponding Day 10 nutrient concentration measurements and final algal mass were covariates.

Conclusions

My dissertation investigated the role of two invasive species, a mud snail and the parasite that uses it, in a highly disturbed, eutrophic estuary. To successfully manage and restore coastal systems like Elkhorn Slough, we need to understand how they process anthropogenic nitrogen, and characterizing the roles that dominant grazers like *Batillaria* play is essential to this. As a whole, my research indicates that all those *Batillaria* and the trematodes that use the snails are having large effects on ecosystem processes. We need to learn more to fully understand the ecological effects of this multi-species invasion, but I have discovered some important information in my research. In Chapter 1, I showed that parasites change the physiology and habitat use of the dominant mud flat grazer in Elkhorn Slough. In Chapter 2, I showed that snails influence the microalgal community on the mud flat, and that these influences are drastically altered by parasite-mediated changes to the snail, which in turn should affect other bottom-dwelling species, and the estuarine food web. Since parasites are so important to these interactions, and parasite productivity is expected to increase along with rising global temperatures, we must take them into account in food web modeling and ecosystem-based management. In Chapter 3, I showed that *Batillaria* could be to making seasonal macroalgal blooms worse by fertilizing the algae, increasing its growth and nutrient status. Together, my results, underscore the context-dependency of species interactions, and demonstrate that parasites, along with their hosts, must be considered in managing important habitats like Elkhorn Slough.

APPENDIX I: INITIAL AND FINAL ALGAL METRICS

A: Algal mass ± SE

Snail Treatment	NO_3^- Treatment	mean initial algal mass ± SE (g)	mean final algal mass ± SE (g)	mean % Δ algal mass ± SE
Contact	Low	0.85 ± 0.04	0.95 ± 0.04	+12.77 ± 4.12
Caged	Low	0.82 ± 0.03	1.02 ± 0.03	+25.40 ± 3.98
Control	Low	0.85 ± 0.04	1.03 ± 0.08	+21.73 ± 7.84
Contact	High	0.86 ± 0.05	1.04 ± 0.05	+20.94 ± 2.41
Caged	High	0.77 ± 0.05	1.00 ± 0.08	+28.54 ± 3.70
Control	High	0.89 ± 0.06	0.94 ± 0.07	+7.57 ± 8.06

B: Algal %N ± SE

Snail Treatment	NO_3^- Treatment	mean initial %N ± SE	mean final %N ± SE	mean Δ %N ± SE
Contact	Low	2.37 ± 0.12	2.17 ± 0.12	- 0.20 ± 0.16
Caged	Low	2.64 ± 0.10	2.26 ± 0.07	- 0.38 ± 0.08
Control	Low	2.45 ± 0.13	1.76 ± 0.09	- 0.69 ± 0.13
Contact	High	2.48 ± 0.10	2.54 ± 0.16	+0.06 ± 0.22
Caged	High	2.56 ± 0.06	2.30 ± 0.08	- 0.26 ± 0.10
Control	High	2.48 ± 0.11	2.01 ± 0.15	- 0.47 ± 0.20

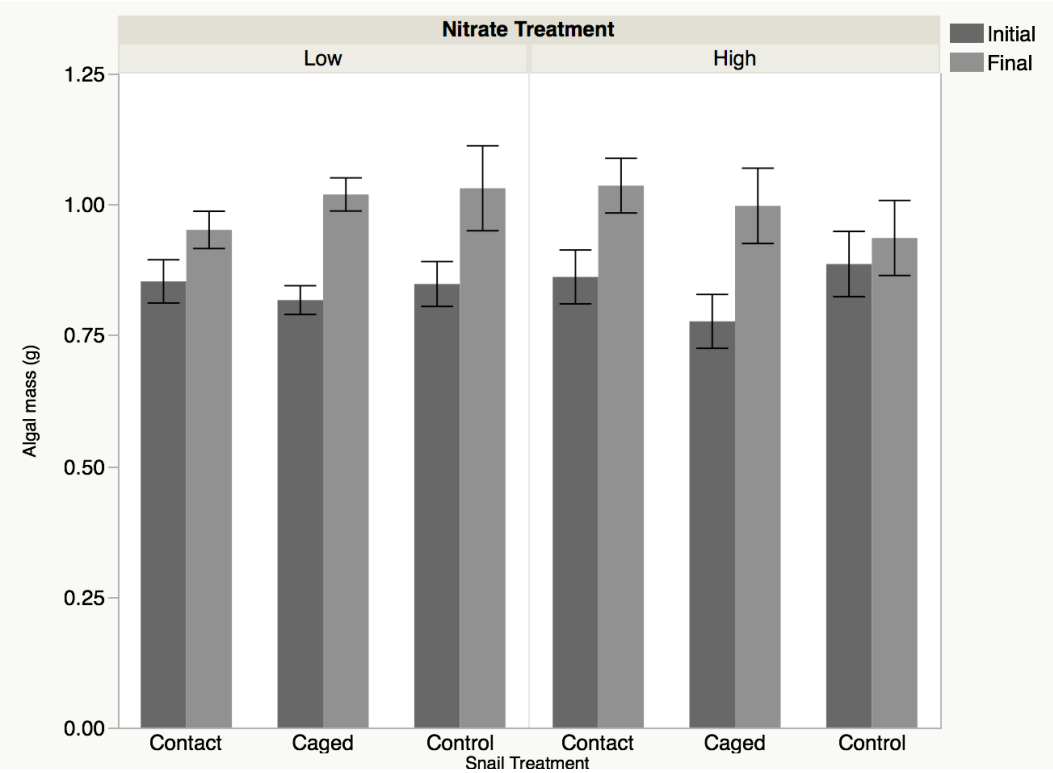
C: Algal C/N ± SE

Snail Treatment	NO_3^- Treatment	mean initial C/N ± SE	mean final C/N± SE	mean Δ C/N± SE
Contact	Low	11.63 ± 0.59	12.95 ± 0.48	+ 1.32 ± 0.61
Caged	Low	10.64 ± 0.28	13.44 ± 0.32	+ 2.80 ± 0.32
Control	Low	11.85 ± 0.54	14.90 ± 0.60	+ 3.05 ± 0.41
Contact	High	10.72 ± 0.22	11.37 ± 0.48	+ 0.65 ± 0.58
Caged	High	10.66 ± 0.17	13.25 ± 0.37	+ 2.59 ± 0.35
Control	High	10.66 ± 0.24	13.98 ± 0.76	+ 3.32 ± 0.86

D: Algal yield ± SE

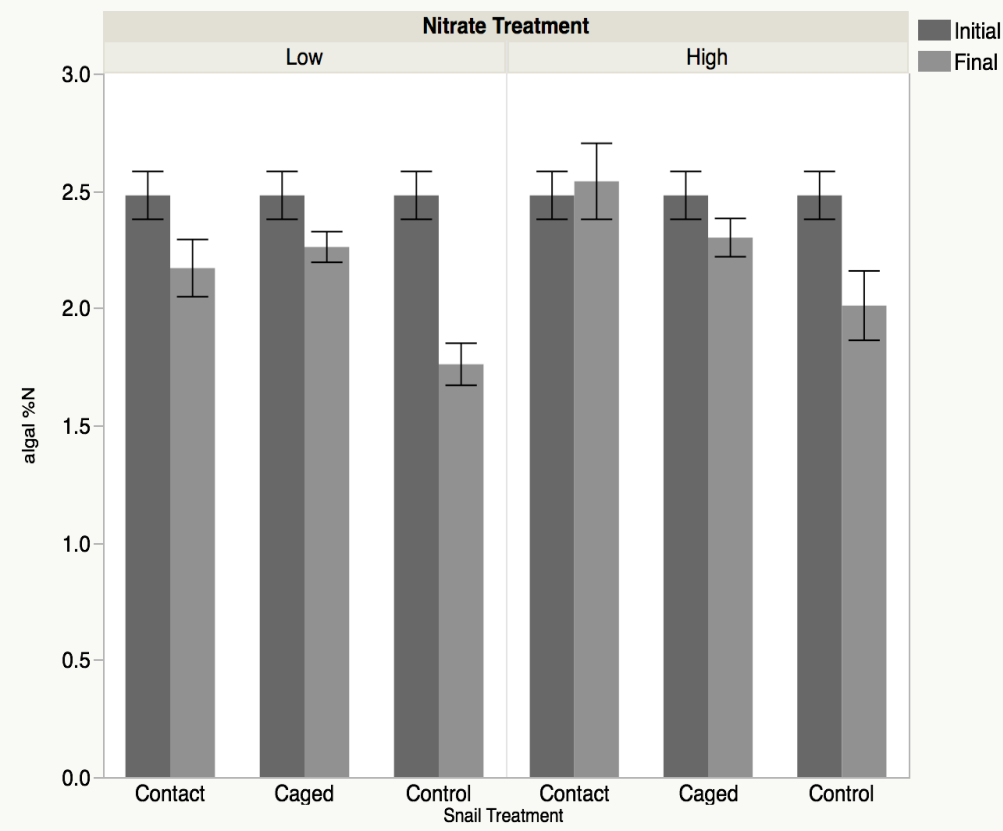
Snail Treatment	NO_3^- Treatment	mean initial algal yield ± SE	mean final algal yield ± SE	mean % Δ algal yield ± SE
Contact	Low	0.757 ± 0.004	0.695 ± 0.010	- 0.061 ± 0.013
Caged	Low	0.749 ± 0.003	0.675 ± 0.011	- 0.077 ± 0.011
Control	Low	0.756 ± 0.002	0.661 ± 0.010	- 0.096 ± 0.011
Contact	High	0.753 ± 0.003	0.698 ± 0.011	- 0.056 ± 0.010
Caged	High	0.750 ± 0.004	0.684 ± 0.008	- 0.067 ± 0.008
Control	High	0.754 ± 0.005	0.695 ± 0.005	- 0.059 ± 0.006

E: Mean initial and final algal mass



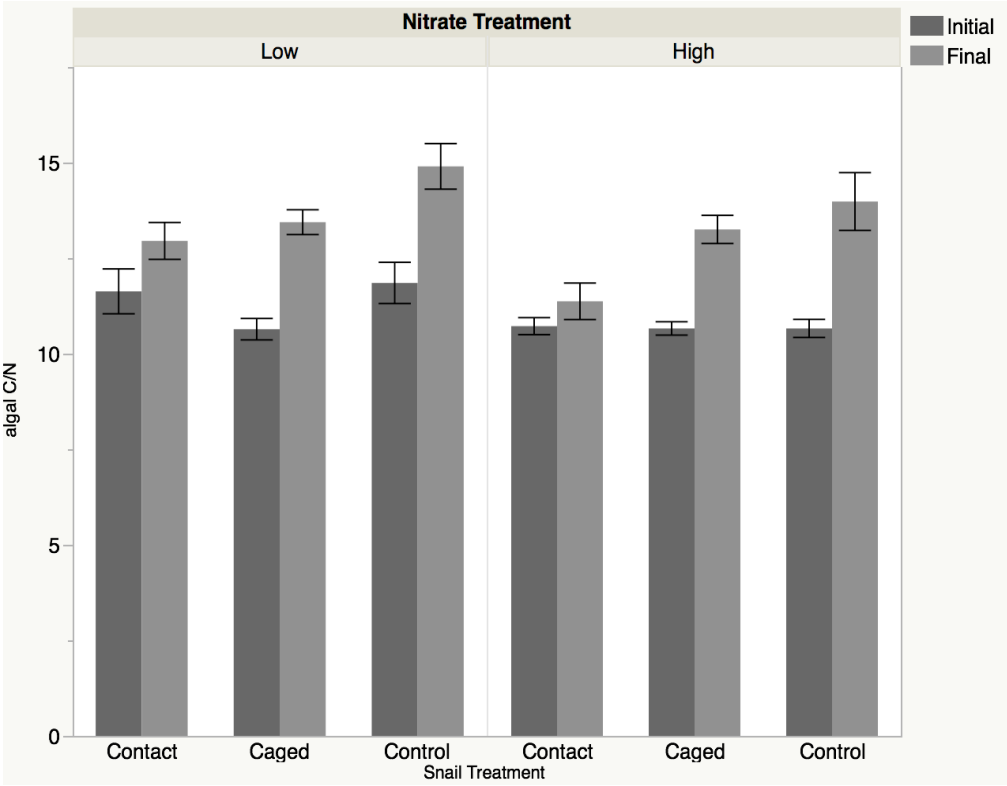
Mean initial and final algal mass with standard error bars. The left pane shows mean algal mass by snail treatment in low nitrate; the right pane shows mean algal mass by snail treatment in high nitrate.

F: Initial and final algal %N ± SE



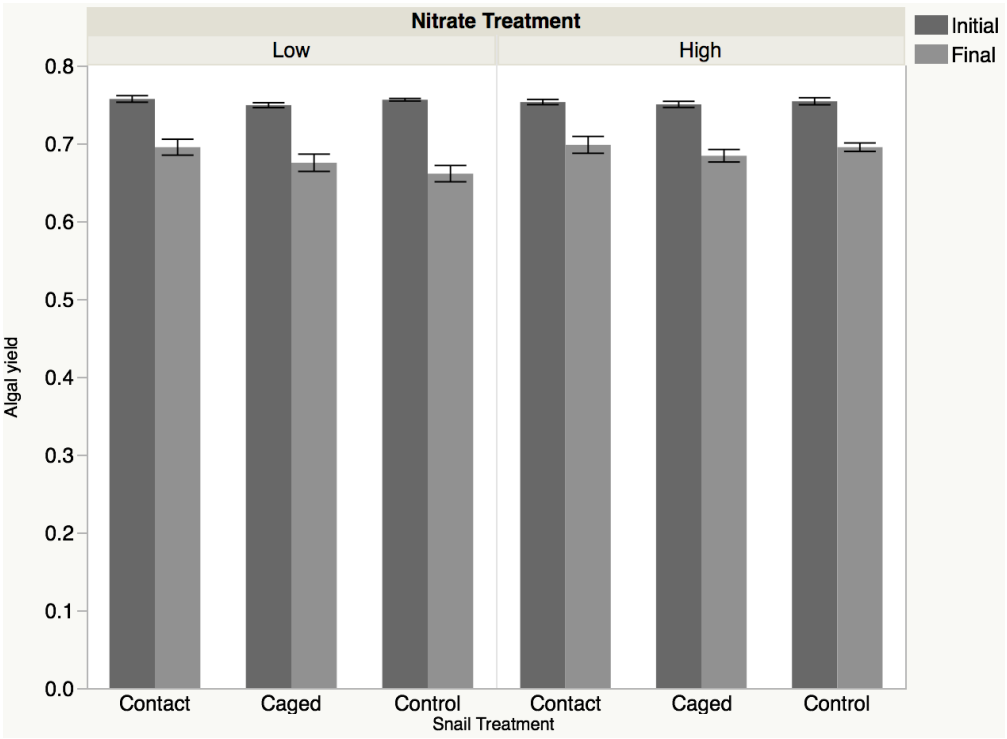
Mean initial and final algal %N with standard error bars. The left pane shows mean algal %N by snail treatment in low nitrate; the right pane shows mean algal %N by snail treatment in high nitrate.

G: Initial and final algal C/N ± SE



Mean initial and final algal C/N with standard error bars. The left pane shows mean algal C/N by snail treatment in low nitrate; the right pane shows mean algal C/N by snail treatment in high nitrate.

H: Initial and final algal yields ± SE



Mean initial and final algal photosynthetic yield with standard error bars. The left pane shows mean photosynthetic yield by snail treatment in low nitrate; the right pane shows mean photosynthetic yield by snail treatment in high nitrate.

APPENDIX II: WATER CHEMISTRY TABLES AND FIGURES

A: Experimental water [NOx]

Snail Treatment	NO_3^- Treatment	Day 6 ± SE (µM)	Day 10 ± SE (µM)	treatment water (µM)
Contact	Low	1.12 ± 0.09	1.30 ± 0.13	8.04
Caged	Low	0.79 ± 0.11	1.52 ± 0.15	8.04
Control	Low	0.97 ± 0.07	1.33 ± 0.12	8.04
Contact	High	1.00 ± 0.11	1.51 ± 0.07	51.30
Caged	High	1.02 ± 0.12	1.26 ± 0.09	51.30
Control	High	1.15 ± 0.06	1.14 ± 0.11	51.30

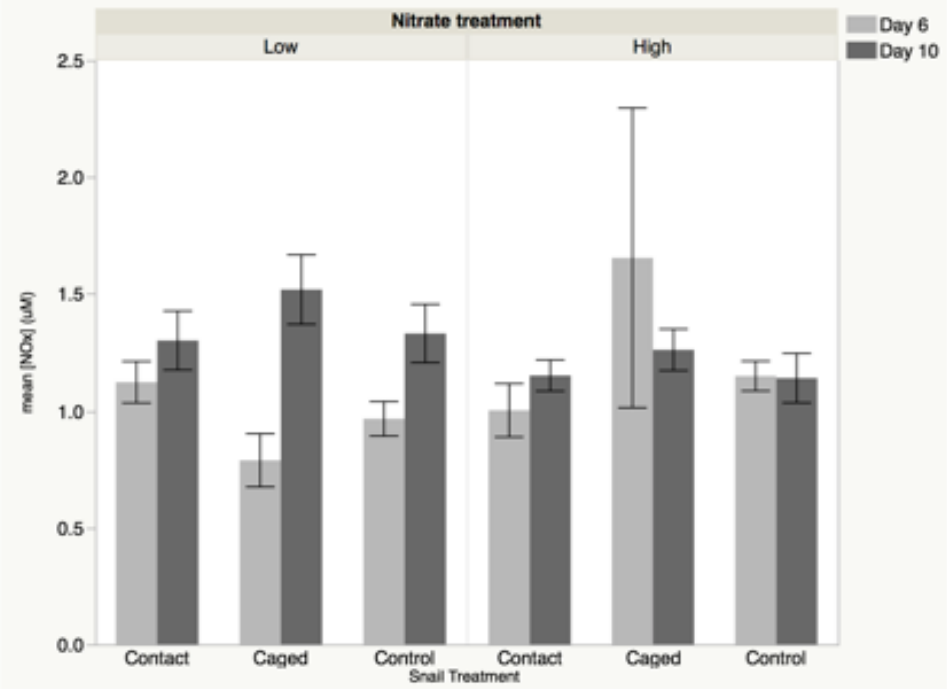
B: Experimental water [PO_4^{3-}]

Snail Treatment	NO_3^- Treatment	Day 6 ± SE (µM)	Day 10 ± SE (µM)	treatment water (µM)
Contact	Low	-0.03 ± 0.02	0.00 ± 0.02	-0.10
Caged	Low	1.07 ± 0.14	1.27 ± 0.15	-0.10
Control	Low	0.22 ± 0.09	0.01± 0.02	-0.10
Contact	High	0.08 ± 0.04	0.00 ± 0.03	-0.11
Caged	High	0.89 ± 0.25	0.70 ± 0.13	-0.11
Control	High	0.19 ± 0.11	-0.04 ± 0.02	-0.11

C: Experimental water [NH_4^+]

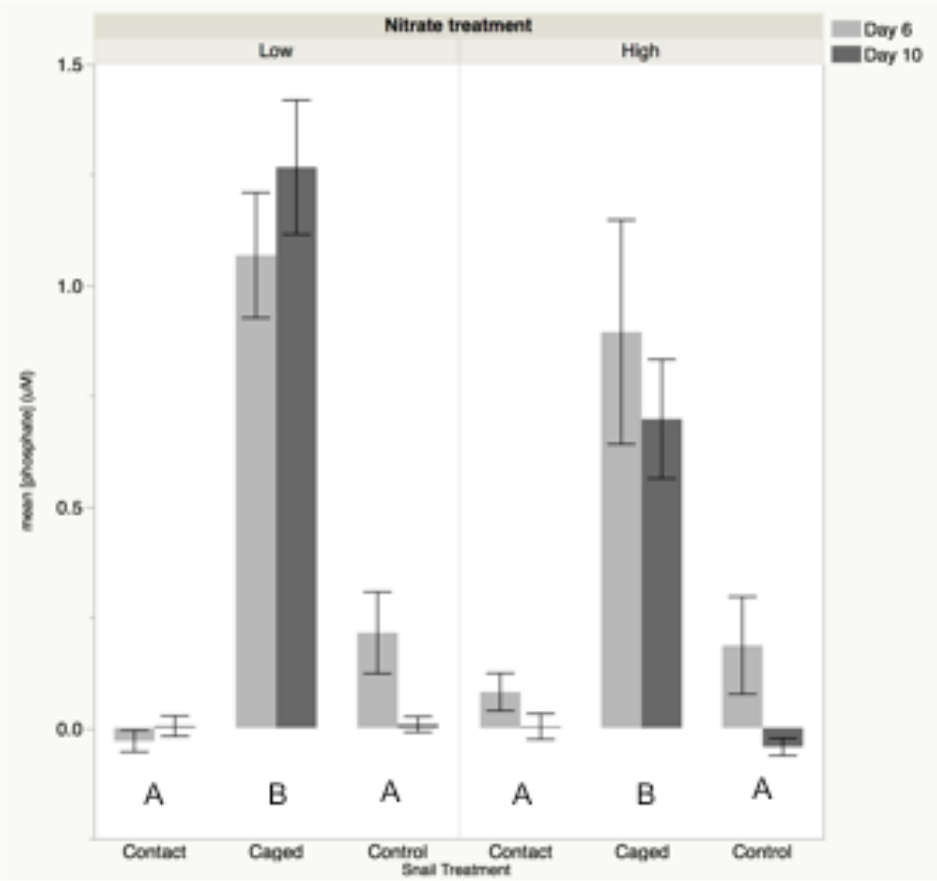
Snail Treatment	NO_3^- Treatment	Day 6 ± SE (µM)	Day 10 ± SE (µM)	treatment water (µM)
Contact	Low	1.63 ± 0.23	1.69 ± 0.41	0.96
Caged	Low	1.26 ± 0.27	5.83 ± 1.92	0.96
Control	Low	1.03 ± 0.35	0.49 ± 0.12	0.96
Contact	High	1.00 ± 0.11	1.51 ± 0.09	1.41
Caged	High	0.72 ± 0.20	1.32 ± 0.37	1.41
Control	High	0.40 ± 0.26	0.36 ± 0.09	1.41

D: Mean [NOx] in experimental chambers ± SE



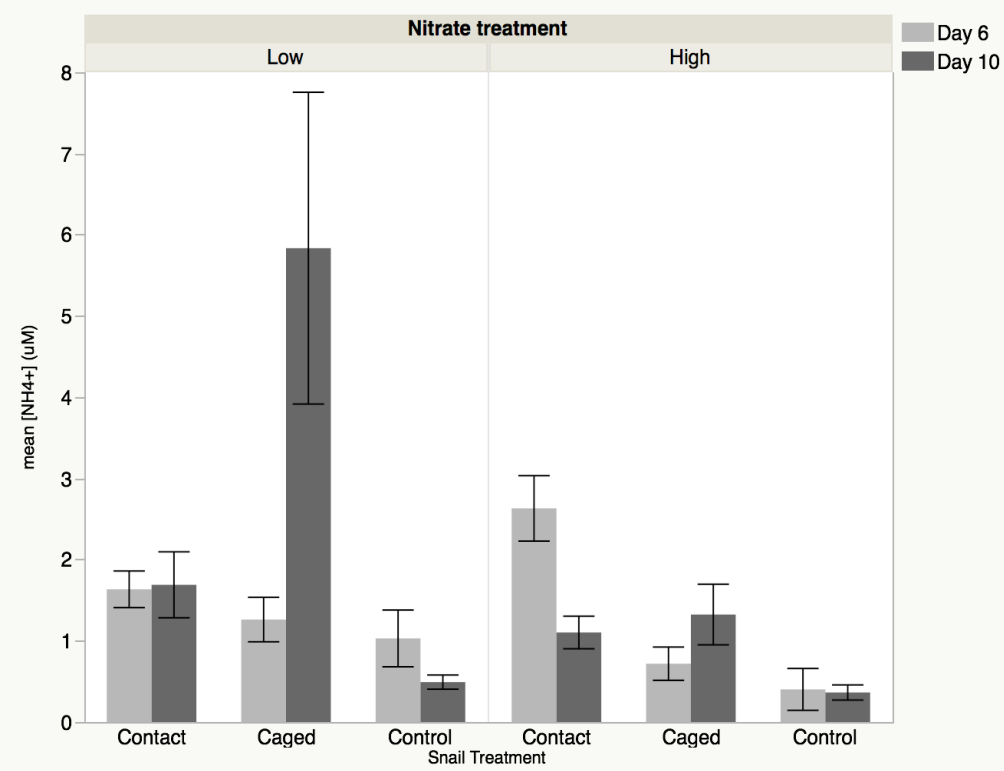
Mean [NOx] in experimental chambers on Days 6 and 10 of the 10-day experiment. Water was collected from microcosms during water changes; water collected on Day 6 and Day 10 was put in experimental chambers since Days 4 and 8, respectively; [NOx] refers to the concentration in experimental chambers after ~48 h. The left panel shows mean [NOx] for experimental chambers for the low nitrate (8.04 μM) treatment; the right panel shows mean [NOx] for experimental chambers for the high nitrate (51.30 μM) treatment. Error bars show standard errors.

E: Mean $[PO_4^{3-}]$ ± SE in experimental chambers



Mean $[PO_4^{3-}]$ in experimental chambers on Days 6 and 10 of the 10-day experiment. Error bars show standard error. Water was collected from microcosms during water changes; water collected on Day 6 and Day 10 was put in experimental chambers on Days 4 and 8, respectively. $[PO_4^{3-}]$ refers to the concentration in experimental chambers after ~48 h. The left panel shows mean $[PO_4^{3-}]$ for experimental chambers in the low nitrate (8.04 µM) treatment; the right panel shows mean $[PO_4^{3-}]$ for experimental chambers for the high nitrate (51.30 µM) treatment. Different letters above bars indicate statistically ($p \leq 0.05$) different changes in experimental water $[PO_4^{3-}]$ among snail treatments in separate factorial ANCOVAs run for Day 6 and Day 10. The Day 6 analysis was run using Day 6 $[PO_4^{3-}]$ as the response variable, with snail and nitrate treatments as factors and Day 6 [NOx], Day 6 $[NH_4^+]$, and initial algal mass as co-variates. For the Day 10 analysis, the same factors were used, with Day 10 [NOx], Day 10 $[NH_4^+]$, and final algal mass as covariates. Snail treatment significantly affected $[PO_4^{3-}]$ on both days, and Tukey's HSD indicated the same differences among treatments on both Days, so letters marking significant differences are included only once for simplicity.

F: Mean [NH_4^+] ± SE in experimental chambers



Mean [NH_4^+] in experimental chambers on Days 6 and 10 of the 10-day experiment. Error bars show standard error. Water was collected from microcosms during water changes; water collected on Day 6 and Day 10 was put in experimental chambers on Days 4 and 8, respectively; [NH_4^+] refers to the concentration in experimental chambers after ~48 h. The left panel shows mean [NH_4^+] for experimental chambers for the low nitrate (8.04 μM) treatment; the right panel shows mean [NH_4^+] for experimental chambers for the high nitrate (51.30 μM) treatment.

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